Chapter 3
GTPases Rac1 and Ras Signaling from Endosomes

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Abstract The endocytic compartment is not only the functional continuity of the plasma membrane but consists of a diverse collection of intracellular heterogeneous complex structures that transport, amplify, sustain, and/or sort signaling molecules. Over the years, it has become evident that early, late, and recycling endosomes represent an interconnected vesicular-tubular network able to form signaling platforms that dynamically and efficiently translate extracellular signals into biological outcome. Cell activation, differentiation, migration, death, and survival are some of the endpoints of endosomal signaling. Hence, to understand the role of the endosomal system in signal transduction in space and time, it is therefore necessary to dissect and identify the plethora of decoders that are operational in the different steps along the endocytic pathway. In this chapter, we focus on the regulation of spatiotemporal signaling in cells, considering endosomes as central platforms, in which several small GTPases proteins of the Ras superfamily, in particular Ras and Rac1, actively participate to control cellular processes like proliferation and cell mobility.

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3.1 Introduction

3.1.1 An Overview of the Endosomal System

Endocytosis describes the internalization of nutrients, receptor–ligand complexes, fluids, lipids, extracellular proteins and viruses, and many other biomolecules. Once inside cells, endosomes are responsible for the fine-tuning of multiple pathways that enable the degradation, recycling, storage, activation, or signaling of incoming molecules. Since their discovery in the late 70s, using biochemical analysis and cellular fractionation techniques on extracts from rat liver (Bergeron et al. 1978; Posner et al. 1980; Marsh et al. 1983; Debanne et al. 1982), the knowledge on the complexity of the endosomal compartment has vastly increased. Over the years, a huge number of publications revealed an astonishing complex system of vacuolar-tubular endosomal structures with distinct characteristics that are dynamically interconnected, communicating with the plasma membrane and other cellular compartments like the trans-Golgi network (TGN). The proper functioning of this endocytic system guarantees balanced cellular homeostasis and essential cellular processes like proliferation, migration, invasion, division, polarity among many others (Scita and Di Fiore 2010; Huotari and Helenius 2011; Gould and Lippincott-Schwartz 2009).

The endosomal membrane system consists of several different compartments. Early endosomes (EEs) are the first and main sorting station in the endocytic pathway where endocytosed molecules that enter the cell via clathrin-dependent (CDE) or clathrin-independent (CIE) pathways are routed to different cellular destinations. From EEs, most internalized molecules are recycled back to the plasma membrane directly by transport vesicles or indirectly through passage via recycling endosomes (REs). Molecules in EE that are neither segregated for recycling nor directed to the TGN, where it can be delivered to the cell surface via the secretory pathway, are destined for degradation along the lysosomal pathway. For this to occur, EEs mature to late endosomes (LEs)/multivesicular bodies (MVBs) and thereafter fusion with lysosomes (Scita and Di Fiore 2010; Huotari and Helenius 2011; Mayor and Pagano 2007). There is also a continuous exchange between the TGN and the EEs in order to direct acid hydrolases from the Golgi apparatus to lysosomes. The structure, identity, and functionality of each endosomal compartment is characterized by a specific luminal pH regulated by the vacuolar proton pump V-ATPase and most relevant to this review, a specific combination of proteins, lipids, and signaling complexes attached to its cytosolic membrane surface. In particular, the family of Rab GTPases is critical for the organization of microenvironments that determine endosomal functions. Rab proteins define the identity of endosomal subdomains by recruiting effectors and facilitate membrane flux along the endocytic pathway (Jovic et al. 2010; Zerial and McBride 2001). In the following, we will briefly outline the characteristics of the various endosomal compartments, their respective Rab proteins and the Rab effectors that define their identity. Intruigingly, some of these proteins and lipids listed below and required to determine uniqueness and guiding of vesicle transport within the endosomal compartment are intimately linked to the trafficking and signaling of rat

sarcoma (Ras) and Ras-related C3 botulinum toxin substrate 1 (Rac1) proteins from EEs, REs, and LEs (see Sect. 3.1.2 onwards).

EEs represent a weakly acidic (pH 6.8–6.1) compartment that is mainly located in the periphery of the cell. This compartment is principally characterized by a tubulo-vesicular morphology and the presence of Rab5 and its effector Vps34, a phosphatidylinositol 3-kinase type III that generates the phosphoinositide (PI) PtdIns(3)P. The presence of active Rab5 (Rab5-GTP), which is generated in a guanine exchange factor (GEF) [rabaptin 5-associated exchange factor for RAB5 (Rabex5)] and PtdIns(3)P-dependent manner, allows the recruitment of the effector early endosome antigen 1 (EEA1), which together with SNAREs (syntaxin 6 and 13) enables endosome fusion. Rab5-GTP and PtdIns(3)P also recruit rabenosin-5, which interacts with EH domain-containing protein 1 (EHD1) and regulates recycling from EEs. In addition, EEs contain distinctive membrane microdomains enriched in specific proteins [including Rab4, Rab11, ADP ribosylation factor 1 (Arf1), complex protein I (COPI), retromer, Rab9, or Rab7] that together with the cytoskeleton, actin, and microtubules, regulate the subcellular trafficking of certain cargoes through slow and fast recycling routes to the plasma membrane, the degradative pathway to lysosomes or retrograde transport to the TGN (Huotari and Helenius 2011; Vonderheit and Helenius 2005; Rojas et al. 2008; Bonifacino and Rojas 2006; Hayer et al. 2010; Bonifacino and Hurley 2008; Pfeffer 2009; Zerial and McBride 2001; Sigismund et al. 2012; Johannes and Popoff 2008). There is also a subpopulation of EEs that instead of EEA1 contain the Rab5 effector Adaptor protein containing PH domain, PTB domain, and Leucine zipper motif 1/2 (APPL1/2). As outlined below, the presence of APPL1/2 on EE might be responsible to create specific endosomal subpopulations that can trigger signaling events related to cell growth (see Sect. 3.1.2) (Miaczynska et al. 2004; Schenck et al. 2008; Zoncu et al. 2009).

Rab5 also promotes the transformation of EEs to LEs by ensuing conversion from a Rab5-positive to a Rab7-positive (LE) compartment. This Rab conversion is achieved when the Mon1-complex binds to PtdIns(3)P and facilitates the exchange of the Rab5-GEF Rabex5 for the Rab7-GEF that is associated with the homotypic fusion and protein sorting (HOPS) complex (Poteryaev et al. 2010; Rink et al. 2005). In this LE maturation, intraluminal vesicles (ILVs) are acquired through the participation of the endosomal sorting complex required for Transport (ESCRT) complexes by a regulated posttranslational ubiquitination and de-ubiquitination modification. Although a simple maturation that confers the conversion of EE to LE compartment is possible, it seems that a fission of domains within EE that acquired LE features participates in the formation of the LE compartment. The resulting endosomal carrier vesicles (ECVs) from this fission process move to the center of the cell via microtubules and fuse with the LE compartment. The vacuolar LEs, arranged in a perinuclear location, are more acidic (pH: 6.0–5.0), contain Rab7 and the PtdIns(3,5)P_2, which is synthesized by the PtdIns(3)P 5-kinase FAB1/PIKfyve. Finally, LEs will mature or fuse

with lysosomes containing acidic hydrolases (proteases and lipases among others). This compartment has a pH around 5.0–4.5, and its membrane is protected by the presence of lysosome-associated membrane proteins (LAMP) proteins (Huotari and Helenius 2011; Poteryaev et al. 2010; Jovic et al. 2010; Scott et al. 2014; Platta and Stenmark 2011).

3.1.2 Endocytosis and Signaling from Endosomal Compartments

Over the last decades, it has become apparent that signaling within the endosomal system contributes to an enormous variety of events that participate in a range of cellular processes. This has led to the widely accepted concept of the "signaling endosome."

This concept recognizes endocytosis to play a key role attenuating signals generated by activated receptors at the plasma membrane, directing them into the degradative-lysosomal pathway. On the other hand, on route to lysosomes, these active receptors can continue to signal in EEs and LEs/MVBs. Therefore, endosomes act as signaling platforms to maintain or prolong signals generated at the plasma membrane. Sustained stimulation can be also enhanced by increased recycling of receptors to the plasma membrane, which is often observed in tumor cells in order to intensify proliferative signals. In addition, endosomes also seem to promote localized and selective recruitment of scaffold and effectors proteins, thereby assembling specific modules. These signaling building blocks can also be transported and directed, through endocytosis-mediated recycling, to specific places in the cell or to specific domains at the plasma membrane to achieve functionality (Sigismund et al. 2012; Lobert and Stenmark 2011; Jones et al. 2006; Frittoli et al. 2011; Taub et al. 2007; Teis and Huber 2003; Palamidessi et al. 2008; White et al. 2006; Sorkin and von Zastrow 2009; Platta and Stenmark 2011; Ohashi et al. 2011; Puthenveedu et al. 2010; Dobrowolski and De Robertis 2011; Schiefermeier et al. 2014; Villasenor et al. 2016).

A substantial number of publications, using biochemical cellular fractionation techniques as well as new live cell imaging techniques such as fluorescence resonance energy transfer (FRET), fluorescence-lifetime imaging microscopy (FLIM), photoactivatable fluorescent proteins (PAFPs), or Ras and interacting protein chimeric unit (Raichu) biosensors (Gonnord et al. 2012; Miaczynska and Bar-Sagi 2010; Murphy et al. 2009), have highlighted the role of the endocytic system to attenuate or sustain signaling, to participate in specific outcomes and to direct modules or complexes to specific subcellular sites or microdomains. The potential relevance of the endocytic system is highlighted in cancer, where altered dynamics of the endocytic pathway are often associated with an inability to properly internalize, recycle, or degrade key cancer drivers such as receptor tyrosine kinases, leading to aberrant proliferation

and metastasis of tumor cells (Stasyk and Huber 2016; Lanzetti and Di Fiore 2008; Porther and Barbieri 2015).

Before embarking on the focus of this chapter, the signaling of the small GTPases Ras and Rac on endosomes (Sect. 3.2), it is essential to outline the overarching role of cell surface receptors in signal transduction. Hence, in the following, we will first illustrate in more detail the endosome signaling outputs for some receptors.

Specific endosomal signaling has been demonstrated for several families of cell surface receptors, in particular, receptor tyrosine kinases (RTKs) such as the epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), hepatocyte growth factor receptor (HGF-R or Met), tropomyosin receptor kinase A (TrkA), and insulin receptor (Ins-R). In addition, serine/threonine kinases such as transforming growth factor beta (TGFβ) receptors, G-protein coupled receptors (GPCRs), or Wnt and Notch receptors have also been reported to signal from endosomes (Stasyk and Huber 2016; Joffre et al. 2011; Wang et al. 2002; Sorkin and von Zastrow 2009; Dobrowolski and De Robertis 2011; Kermorgant and Parker 2008; Murphy et al. 2009; Flinn et al. 2010; Gould and Lippincott-Schwartz 2009; Lanzetti and Di Fiore 2008; Le Roy and Wrana 2005; Tomas et al. 2014; Mellman and Yarden 2013; Barrow-McGee and Kermorgant 2014).

To reach the endosomal compartment, the above-mentioned receptors appear to employ different internalization routes, either via CDE or CIE. Interestingly, depending on ligand concentration, some receptors can be internalized by both entry routes, which then differentially impacts on their signaling output. For instance, while CIE for TGFβ-R and EGFR directs ligand–receptor complexes to lysosomes for degradation, the CDE routes these receptors to signaling competent endosomal vesicles distinct from the lysosomal pathway (Sigismund et al. 2008, 2012). Moreover, signaling of these TGFβ- and EGF receptors, that remain ligand-bound and active after internalization, are principal examples for the creation of a very localized and specific signal transduction elicited from EE microdomains exclusively located within a population of Smad anchor for receptor activation (SARA)- and APPL-positive endosomes, respectively. SARA, which binds PtdIns(3)P on EEs, is a scaffold protein that interacts with TGFβ-R and Mothers against decapentaplegic (SMAD) family member 2 enabling the phosphorylation of the former by TGFβ and its translocation to the nucleus to regulate gene transcription (Hayes et al. 2002; Tsukazaki et al. 1998; Di Guglielmo et al. 2003). On the other hand, APPL, which recruits protein kinase B (PKB or AKT) and its substrate glycogen synthase kinase 3 (GSK3), is activated and translocated to the nucleus by the endocytosed EGFR (Miaczynska et al. 2004).

Other examples for the relevance of endosomal signaling establishing discrete cellular functions include the sustained signaling of GPCRs on endosomes, which is important to ensure continuous cyclic AMP production and mitogen-activated protein kinase (MEK)—extracellular signal-regulated kinases (ERK) signaling beyond its initial activation at the cell surface (Vilardaga et al. 2014; Shenoy and Lefkowitz 2011; Sorkin and von Zastrow 2002). Internalization of nerve growth factor (NGF) bound to its TrkA receptor has also been proven necessary to prolong activation of Rap1 GTPase and to promote neuronal survival via ERK5 activation and cAMP response element binding protein (CREB)-dependent transcription (Wu et al. 2001;

Watson et al. 2001). In addition, the NGF-TrkA receptor–ligand complex can also activate the Ras/mitogen-activated protein kinase (MAPK) pathway from endosomes in neuronal cells (Howe et al. 2001).

EGFR exemplifies a classical example of specific and sustained signaling on endosomes. EGF induces the accumulation of EGFR and downstream signaling molecules such as SH2-containing collagen related (Shc), growth factor receptor-bound protein 2 (Grb2), son of sevenless (Sos), Ras, as well as the serine/threonine kinases rapidly growing fibrosarcoma protein 1 (Raf-1), Mek, and Erk1/2 in endosome compartments (Balbis et al. 2007; Lu et al. 2009; Moreto et al. 2008; Pol et al. 1998; Sorkin and von Zastrow 2009; Teis et al. 2006; Di Guglielmo et al. 1994; Nada et al. 2009; Teis et al. 2002; Wang et al. 2002). In fact, early pioneering work from Vieira et al. elegantly demonstrated the importance of CDE for the control of the EGFR signaling cascade (Vieira et al. 1996). In these studies, inhibition of EGFR internalization, through overexpression of a dominant-negative mutant of the vesicular fission protein dynamin (Dynk44A), drastically interfered with activation of Raf-1 kinase downstream Ras and consequently MAPK signal output, indicating a crucial role of endosomes in the EGFR/Ras/Raf-1/MAPK signaling cascade (Vieira et al. 1996; Moreto et al. 2008). Furthermore, in elegant experiments from Wang et al., the EGFR tyrosine kinase inhibitor AG1478 was used to inhibit EGFR signaling at the cell surface, leading to the internalization of nonactive EGF-EGFR complexes into endosomes. Subsequent removal of AG1478 then enabled activation of endosome-associated EGFR followed by Ras, ERK1/2, and Akt signaling to promote cell proliferation and survival (Wang et al. 2002).

Under certain conditions, EGFR signaling likely occurs in the LE compartment, as the late endosomal adaptors p14 and Mek partner-1 (MP1) can recruit Mek1 to participate in EGFR-induced MAPK activation (Teis et al. 2002, 2006). Indeed, in primary hepatocytes late endosomal EGFR signaling has recently been demonstrated to participate in cell cycle progression (Luo et al. 2011). The p14/MP1 complex also serves as a scaffold to recruit Rag GTPases, which sense amino acid levels and together with Ras homolog enriched in brain (RheB) and hVps34, activate mammalian target of rapamycin complex 1 (mTORC1) in the endo-lysosomal (LE/Lys) compartment to promote cell growth via protein synthesis (Sancak et al. 2010; Zoncu et al. 2011; Duan et al. 2015).

In addition, p14/MP1 in the LE/Lys compartment is also critical for cell migration, as the adaptor complex is directed to the cell periphery, in a Rab7-regulated manner, in order to promote focal adhesion (FA) turnover for cell motility and tumor invasion (Schiefermeier et al. 2014). Furthermore, in the context of cell migration, EEs, and LE/Lys have also been identified to facilitate receptor-mediated signaling events that activate and transport Rac1 GTPase to the leading edge (Palamidessi et al. 2008; Menard et al. 2014; Joffre et al. 2011). Specific guanine exchange factors (GEFs) of the small GTPase Rac1 are known to activate Rac1 in EEs and LEs. This is followed by vesicular transport to the plasma membrane, where active Rac1 then regulates the actin cytoskeleton to promote the formation of lamellipodia in the leading edge, altogether creating forward movement. In the rear of migratory cells, EE and LE control the delivery of the pro-migratory Endo180 receptor to promote adhesion

disassembly by the Rho kinase-derived contractile signals (Gould and Lippincott-Schwartz 2009; Sturge et al. 2006).

Further to the trafficking routes of Rac1 and Endo180 receptor to the front and rear of moving cells, respectively, several recent publications have highlighted the importance of recycling for the delivery of integrins from endosomes to specific plasma membrane domains, which in combination with the export of membrane type-1 matrix metalloproteinase 1 (MT1-MMP) also regulates motility and invasiveness (De Franceschi et al. 2015; Alanko and Ivaska 2016). Along these lines, impaired cholesterol export from LEs in Niemann–Pick type C1 (NPC1) mutant cells or upon overexpression of annexin A6 interfered with the task of the RE compartment to deliver cargo to the cell surface. This imbalanced distribution of intracellular cholesterol strongly reduced integrin recycling from RE to plasma membrane, and consequently, inhibited cell migration (Garcia-Melero et al. 2016; Reverter et al. 2014). Integrins are transmembrane adhesion proteins that by forming FAs connect the F-actin cytoskeleton to the extracellular matrix (ECM), thus attaching cells to their surroundings. In migrating cells, integrin recycling via a fast and Rab4-dependent, as well as a slow and Rab11-dependent recycling route, is the driving force to continuously assemble and disassemble FAs in the leading edge enabling forward movement (De Franceschi et al. 2015; Shafaq-Zadah et al. 2016). Additionally, integrin-dependent adhesion to collagen type-1 matrix protein stimulates MT1-MMP translocation, from the intracellular biosynthetic-storage compartment and by a Rab8-dependent exocytosis, to surface structures that promote invasion of MDA-MB-231 tumor cells (Bravo-Cordero et al. 2007). However, additional Rab GTPases (Rab2, Rab5, Rab7, Rab14) and the internalization process could also regulate MT1-MMP activity at the plasma membrane in other settings (Frittoli et al. 2011; Wiesner et al. 2013; Williams and Coppolino 2011; Castro-Castro et al. 2016; Kajiho et al. 2016). Interestingly, from the point of view of endosomal signaling, integrins bind distinct sets of proteins when located at the plasma membrane or in endosomes. Consequently, this elicits different signal output in endosomes: activation of focal adhesion kinase (FAK), Akt, ERK, and suppression of anoikis (Alanko et al. 2015; Alanko and Ivaska 2016).

In summary, in this section we have given an overview how the endo/lysosomal system provides membranous platforms to regulate spatiotemporal signaling in cells (Flinn et al. 2010; Gould and Lippincott-Schwartz 2009; Kermorgant and Parker 2008; Lobert and Stenmark 2011; Ohashi et al. 2011; Palamidessi et al. 2008; Platta and Stenmark 2011; Sorkin and von Zastrow 2009; Taub et al. 2007). In the following, we will discuss in more detail how the endosomal localization of several small GTPases proteins of the Ras superfamily actively participates to control cellular processes like proliferation and cell mobility.

3.2 Signaling of the Small GTPases Ras and Rac1 from Endosomes

The Ras superfamily of GTPases comprising a total of 150 members is also known as small GTPases due to their small molecular weight (20–40 kDa). They are classified into six subfamilies: Ras, Rho, Rab, Arf, Ran, and Galpha subunits (Flinn et al. 2010; Rojas et al. 2012). The Rho family members control cytoskeleton dynamics and cellular mobility, while Rab and Arf proteins modulate formation and transport of intracellular vesicles, including exo- and endocytosis. The Ran family coordinates nuclear transport processes, and Galpha subunits manage GPCR signaling. Finally, Ras GTPases are located upstream signaling cascades such as the Raf-1/MAPK pathway that is known to regulate transcription relevant for proliferation and differentiation processes among others.

Rac1, which will be discussed in more detail in Sect. 3.2.2, belongs to the Rho subfamily and within the Ras subfamily there are four isoforms (HRAS, NRAS, KRAS4A, and KRas4B). All of them have in common a module called the G-domain, which adopts an α/β topology and contains 60–180 residues, responsible for nucleotide (GTP)-dependent conformational changes of two internal regions named switch region I (residues 30–38) and switch region II (residues 59–67) (Wittinghofer and Vetter 2011). The ability to bind GTP and the concomitant conformational changes associated with GTP or GDP binding enables Ras proteins to act as binary molecular switches, active when GTP is bound to the G-domain and inactive when GDP is associated with the G-domain. In the active state (GTP-bound), GTPases interact and activate a plethora of different effectors with different functions, including Raf-1, phosphoinositide 3–kinase (PI3K), RAL guanine nucleotide dissociation stimulator (RalGDS), T lymphoma invasion and metastasis-inducing 1 (TIAM1), WASP-family verprolin-homologous protein (WAVE,) and others. The active and inactive state cycle is controlled by guanine exchange factors (GEFs) which facilitate the exchange of GDP for GTP. The ability of GEFs to remove GDP enables more efficient GTP binding which is found at approximately tenfold higher concentrations compared to GDP in the cytosol. On the other hand, GTPase activating proteins (GAPs) increase the intrinsic GTP hydrolyzing capacities of GTPases to ensure rapid inactivation (Rajalingam et al. 2007; Downward 1996; Marshall 1996). Both, GEFs and GAPs, are spatially and temporally modulated by external stimuli and signaling molecules (Bos et al. 2007).

Given their prominent localization at the plasma membrane and proximity to RTKs and GPCRs, Ras GTPases are key players in the initial steps of signaling cascades generated at the cell surface and therefore control important processes such as proliferation, differentiation, apoptosis, cytoskeleton dynamics or cell motility (Malumbres and Barbacid 2003). However, critical for the "signaling endosome" concept, the presence of Ras proteins in the endosomal compartment makes them the key transducers of the signaling events generated by active receptors along endocytic pathways. As outlined in the following sections in more detail, Ras and Rac1 signaling from endomembranes, in particular from endosomes, have been associated with

proliferation, apoptosis, and/or cellular migration. Nevertheless, it should be noted that some caution should be taken when referring to the conclusions based on Ras and Rac1 signaling from endosomes as the majority of published data has been obtained from model systems that often express unphysiologically high levels of receptors and signaling components.

3.2.1 Ras Signaling from Endosomes

3.2.1.1 The Ras Family

In human cells, Ras isoforms are encoded by three genes: *KRAS* (Kirsten rat sarcoma viral oncogene homolog, *NRAS* (Neuroblastoma RAS viral (v-ras) oncogene homolog), and *HRAS* (Harvey rat sarcoma viral oncogene homolog). This gives rise to 4 different Ras isoforms (~21 kDa): HRas, NRas, KRas4A, and KRas4B (referred to as KRas), the latter two being derived from alternative RNA splicing. Since Ras proteins control proliferation, survival and migration, missense mutations at position G12, G13, and Q61 result in gain-of-function Ras mutants that confer oncogenic activity. All these mutations impair Ras-GTP hydrolysis by inhibiting its intrinsic GTPase activity. Consequently, this interferes with the action of GAPs and Ras remains in a constitutively active state. Hence, oncogenic Ras mutant hyperactivity contributes to the initiation and progression of a large variety of human cancers (~25% of human cancers, with KRas being the most frequently mutated isoform) (Hobbs et al. 2016; Barbacid 1987; Bos 1989; Malumbres and Barbacid 2003; Rajalingam et al. 2007; Newlaczyl et al. 2014).

Given their prominent contribution to the development of tumorigenic events, much effort over the years aimed to unravel the structure, localization and differential function of the Ras isoforms. Structural analysis first revealed that all Ras isoforms shared a highly conserved and nearly identical globular N-terminal domain (residues 1–165), which binds nucleotides and the majority of Ras effectors (see Sects. 3.2.1.3 and 3.2.1.4). However, as described in more detail below, important differences were found in the C-terminal domain (last 24–25 residues), named the hypervariable region (HVR), which is posttranslational modified and responsible for differential subcellular localization and as a result differential signaling among the Ras isoforms (Barbacid 1987; Hancock 2003; Mor and Philips 2006; Fotiadou et al. 2007; Calvo et al. 2010; Eisenberg and Henis 2008; Prior and Hancock 2012). For instance, in cell culture, KRas activates the small GTPase Rac1 more efficiently than HRas because of their differently membrane anchoring and localization, which is consequently translated in more efficient KRas induction of membrane ruffling, pinocytosis, cell motility, and cell survival than HRas (Walsh and Bar-Sagi 2001).

3.2.1.2 Synthesis, Processing, and Trafficking of Ras Isoforms to the Plasma Membrane

Ras proteins are synthesized in the cytosol as globular hydrophilic proteins (188 amino acids in the case of KRas and 189 for all other Ras isoforms) containing a C-terminal CAAX motif (C, cysteine; A, aliphatic residue; X, any residue); being CVLS, CVVM and CVIM for H-, N-, and KRas, respectively. This CAAX sequence is essential for subsequent posttranslational modifications of Ras and its successive targeting to different cellular membranes. Because Ras proteins contain a methionine or a serine as last amino acid (X residue), this CAAX sequence is specifically recognized by a farnesyl transferase in the cytosol, which irreversibly incorporates a farnesyl group (15-carbons isoprenyl) to the cysteine C186, and C185 in KRas. Farnesylation allows Ras proteins to insert and localize on the endoplasmic reticulum membrane for subsequent CAAX modifications which includes AAX hydrolysis by Ras-converting enzyme 1 (Rce1) and the methylation of the remaining farnesylcysteine residue by Isoprenylcysteine carboxyl methyltransferase (Icmt) (Fehrenbacher et al. 2009) (see [1] in Fig. 3.1). Strikingly, despite all these posttranslational modifications occurring for each Ras isoform, H-, N-, and KRas then follow different routes from the ER to the plasma membrane (Mor and Philips 2006).

Although farnesylcysteine methylation is an essential prerequisite, it is not sufficient for cell surface delivery, and Ras needs a second signal to finally reach the plasma membrane or other organelles like endosomes. In the case of KRas, this second signal is a polybasic region (PBR) composed of six basic lysine residues near the farnesylcysteine in the HVR. This PBR motif electrostatically interacts with anionic phospholipids in membranes, such as phosphatidylinositol-4,5-bisphosphate PtdIns (4,5)P_2, the phosphatidylinositol-3,4,5-trisphosphate PtdIns (3,4,5)P_3 or phophatidylserine (PS) (Hancock et al. 1990; Cho et al. 2012; Apolloni et al. 2000; Heo et al. 2006; Yeung et al. 2008; Gelabert-Baldrich et al. 2014). Hence, through a yet still not well-defined Golgi exocytic-independent route that likely involves the above-mentioned electrostatic interactions both signals (farnesyl lipid group and PBR) contribute to direct KRas from the ER to the plasma membrane (Apolloni et al. 2000; Magee and Marshall 1999) (see [2] in Fig. 3.1). The molecular machinery that regulates this trafficking route is also not well known and includes the possibility of a passive electrostatic switch. Alternatively, recent results from the Bastiaens group suggest that the delta-subunit of phosphodiesterase 6 (PDE6-δ), or maybe other chaperone-like proteins, act as a cytosolic solubilization factor and its binding to the farnesyl moiety of KRas could then facilitate KRas trafficking to the plasma membrane. Once at the plasma membrane, the highest electrostatic interaction exerted by acid phospholipids could compete and displace PDE6-δ from KRas, favoring its incorporation or insertion into the inner leaflet of the cell surface phospholipid bilayer (Schmick et al. 2015).

Much different to the complex regulation of KRas translocation to the cell surface, the second signal for targeting H-, N-, and spliced KRas4A proteins to the plasma membrane is represented by the reversible addition of one or two palmitoyl groups at additional cysteine residues adjacent to the CAAX motif. A palmitoyl-transferase

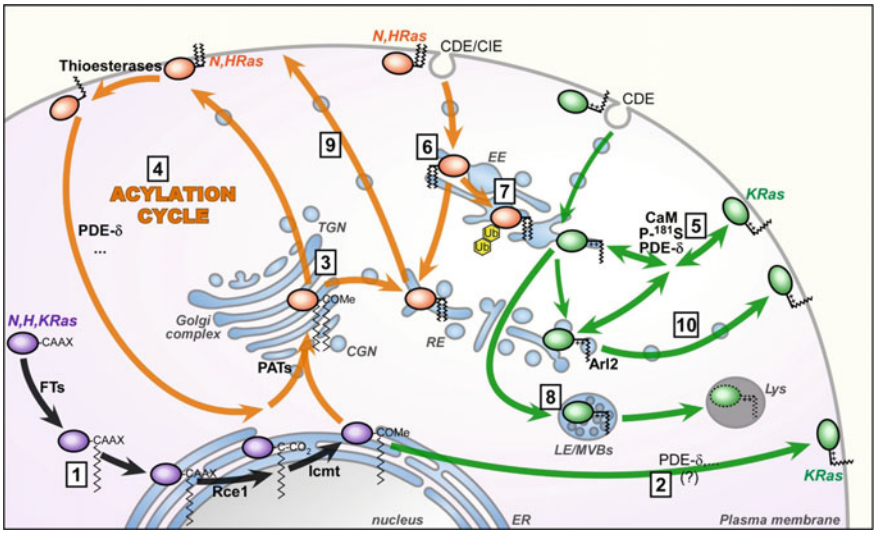

Fig. 3.1 Different routes to the plasma membrane and through the endocytic compartment followed by the Ras isoforms. Once synthesized in the cytosol, Ras isoforms are cysteine farnesylated in their terminal CAAX motif by farnesyl transferases (FTs). This is followed by further posttranslational modifications mediated by Ras-converting enzyme 1 (Rce1) and Isoprenylcysteine carboxyl methyltransferase (Icmt) in the endoplasmic reticulum (ER) [1]. This scheme recapitulates the following traffic routes for the different Ras isoforms: H- or NRas trafficking is shown in orange and KRas trafficking is depicted in green. KRas reaches the PM by still not well-defined non-vesicular routes that likely involve chaperone proteins like PDE-δ [2]. In contrast, H- and NRas use the vesicular exocytic pathway via Golgi and/or Golgi and recycling endosomes (RE) to the plasma membrane (PM) [3]. H- and NRas switch between PM and the Golgi by the action of thioesterases at the PM and palmitoyl-transferases (PATs) at the Golgi, which is known as the acylation cycle [4]. Moreover, from the PM, H-, and NRas can be internalized via CDE or CIE pathways to reach early endosomes (EEs) [6], where they can either be recycled back to PM trough RE [9] or remain retained when ubiquitinated [7]. On the other hand, KRas can also use the vesicular CDE pathway but the bidirectional movement of KRas between PM and endosomal compartments is mainly accomplished through an electrostatic switch modulated by serine phosphorylation (P-^{181}S) or binding proteins, like CaM or PDE-δ, to its hypervariable region [5]. KRas can also return back from RE to the PM via a Arl2-dependent vesicle transport route [10]. In contrast to H- and NRas, KRas moves to late endosome/multivesicular bodies (LE/MVB$_S$) on route for degradation in lysosomes (Lys) [8]

enzyme localized in the Golgi/ER is responsible for palmitoylation of HRas at C181 and C184, NRas at C181and KRas4A at C180 (Hancock et al. 1989; Swarthout et al. 2005). This modification stabilizes the membrane interaction of these Ras isoforms and favors its transport to the plasma membrane through vesicles carriers following the exocytic pathway from the TGN (Choy et al. 1999). In some cases this includes trafficking through REs, which act as a way-station for palmitoylated H- and NRas proteins as they move along the post-Golgi exocytic pathway to the plasma membrane (Misaki et al. 2010) (see [3] in Fig. 3.1). Palmitoylation being

decisive for these Ras proteins to reach the plasma membrane was experimentantlly evidenced in live cell imaging, where green fluorescent protein (GFP)-tagged and palmitoyl-deficient Ras mutants were mislocalized in the ER and Golgi and lacked delivery to the plasma membrane (Hancock et al. 1989; Choy et al. 1999). Note that palmitoylation also contributes to certain levels of diversity in the Ras family. While di-palmitoylated HRas is strongly associated with membranes and can efficiently follow the slow exocytic transport to the plasma membrane, the mono-palmitoylated N- and KRas4A isoforms need additional hydrophobic/basic amino acid sequences (Laude and Prior 2008). The different mechanisms that contribute to Ras processing associated with different trafficking routes are depicted in Fig. 3.1.

One can envisage that the differential contribution of the various CAAX motifs, posttranslational modifications (farnesylation, palmitoylation), together with HVR sequence variations, will impact on their spatiotemporal distribution, with consequences for their ability to activate effector pathways. Hence, the possibility to uncover underlying principles for the creation of signal diversity prompted researchers to extensively examine the distribution of Ras isoforms at the plasma membrane. Indeed, subcellular fractionation as well as advanced imaging identified that once at the plasma membrane, Ras isoforms display distinct localizations in specific membrane subdomains. Moreover, it is now believed that active and inactive Ras proteins are organized in nanoclusters, containing 6–7 Ras proteins per nanocluster. Over the years, it has become clear that these clusters highly depend on the distribution of lipids within the membrane. HRas is the best-characterized member of the Ras family in this context, with HRas-GTP nanoclusters being found in disordered (fluid, cholesterol-poor) domains (Prior et al. 2003; Zhou and Hancock 2015). In contrast, HRas GDP clusters are mainly localized in cholesterol- and sphingolipid-rich domains (lipid rafts). While NRas seems to distribute similar to HRas, KRas is predominantly localized outside lipid rafts. In addition, a distinct cohort of phospholipids, in particular phosphatidylserine, but also phosphatidic acid and phosphatidylinositides contribute to the spatial segregation of Ras isoforms. The different distribution of active and inactive Ras proteins implicates lateral movement of Ras isoforms in the plasma membrane. As Ras activity is determined by GTP/GDP exchange, the localized recruitment and activity of GEFs and GAPs appears critical to ensure the transient nature of Ras nanoclusters (Grewal and Enrich 2006). Interestingly, a specialized form of lipid raft, caveolae, seems to critically translate environmental cues, such as mechanical stress, into the remodeling of lipids at the plasma membrane that in turn extensively modulate the organization of Ras nanoclusters (Ariotti et al. 2014). As such, caveolin-1 deficiency or downregulation of cavin-1, another structural component of caveolae, alters phosphatidylserine distribution at the plasma membrane, which correlates with enhanced KRasG12V nanoclustering and MAPK signaling. Yet lateral segregation of HRas was abolished, thereby compromising signal output from HRasG12V nanoclusters (Ariotti et al. 2014).

In addition to their differential distribution at the cell surface outline above, the different posttranslational modifications of each Ras isoforms affect also the association dynamics of cytosolic Ras protein pools with the plasma membrane. It was

originally postulated that all Ras isoforms display stable membrane association, moving by lateral diffusion as described above (Niv et al. 1999, 2002). However, under certain conditions or upon specific posttranslational modifications, Ras localization at the plasma membrane switches from lateral diffusion to exchange dynamics with cytoplasmic Ras protein pools (Vartak and Bastiaens 2010). As described above, activation of H- and NRas, via GTP-induced conformational changes, leads to their exchange between lipid rafts and non-rafts (Gorfe et al. 2007). Yet, H- and NRas can also dissociate from the plasma membrane in a process known as the acylation cycle, which involves the depalmitoylation of cysteine residues in their HVR domain that is independent of the activation state of Ras (Rocks et al. 2005, 2010). This non-vesicular pathway is mediated by acyl protein thioesterases in the plasma membrane rapidly returning Ras to the diffusing low-membrane affinity farnesylated Ras pool in the cytoplasm. It is yet unclear how the cell translates the intricate network of cellular and environmental signals to provide the balance between lateral diffusion at the plasma membrane or diffusion into the cytosol, but one modulating factor could be the interaction of the Ras-farnesyl group with PDE-δ when Ras proteins are depalmitoylated (Vartak and Bastiaens 2010; Chandra et al. 2011; Goodwin et al. 2005). This could enable protein acetyltransferase (PAT) enzymes to trap depalmitoylated Ras specifically in Golgi/ER membranes, where another round of palmitoylation and trafficking through the exocytic pathway may then be required to ensure the return to the plasma membrane (Rocks et al. 2005; Schmick et al. 2015) (see [4] in Fig. 3.1).

While a general concept of diffusion dynamics for H- and NRas seems to be emerging, the data on trafficking dynamics of KRas to and from the plasma membrane is more complex and in part controversial. Initially, it was proposed that the half-time for KRas membrane desorption was in the order of minutes or even longer, which was in consonance with a postulated stable plasma membrane association regulated by lateral diffusion (Silvius et al. 2006; Niv et al. 1999, 2002). Lately, Silvius et al. (2006), using an inducible heterodimerization technique between ectopically expressed KRas and a complementary binding partner confirmed predominant KRas association with the plasma membrane under basal conditions. Yet, this included a continuous cycling of KRas on and off the membrane in an interval of minutes. Further results from Yokoe and Meyer indicate that the rate of KRas exchange between plasma membrane and cytoplasm may be even more rapid, within seconds (Yokoe and Meyer 1996). In line with these findings, in vitro studies demonstrated that the PBR of KRas associates with lipid bilayers in a rapidly reversible manner with a half-time of seconds or less (Leventis and Silvius 1998). On the other hand, depending on the experimental settings and stimuli, desorption of KRas from the plasma membrane is also modulated by several KRas-interacting proteins, as such as PDE-δ, prenylated Rab protein acceptor protein 1, calmodulin (CaM), and galectin-3 (Chandra et al. 2011; Bhagatji et al. 2010; Elad-Sfadia et al. 2004; Figueroa et al. 2001; Fivaz and Meyer 2005; Lopez-Alcala et al. 2008; Nancy et al. 2002; Villalonga et al. 2001; Philips 2012). In addition, the protein kinase C (PKC)-mediated phosphorylation of active KRas at serine 181 (Ballester et al. 1987) affects the PBR net charge of KRas, thereby inducing an electrostatic switch that displaces KRas from the plasma membrane to endomembranes, including endosomes (Bivona et al. 2006;

Chandra et al. 2011). KRas phosphorylation at serine 181 inhibits CaM binding and accordingly, CaM efficiently interacts with non-phosphorylated KRas-GTP (Lopez-Alcala et al. 2008), an interaction that also favors KRas desorption from the plasma membrane (Bhagatji et al. 2010; Fivaz and Meyer 2005). In this context, elegant studies from Fivaz and Meyer revealed the physiological significance of these interactions in neuronal cells, as cell activation caused the translocation of KRas from the plasma membrane to endosomes through sequestration of the PBR-farnesyl motif in a Ca^{2+}/CaM-dependent manner (Fivaz and Meyer 2005) (see [5] in Fig. 3.1).

3.2.1.3 Ras Trafficking Along Endocytic Routes

Although Ras proteins are predominantly found at the plasma membrane to transduce signals received from surface receptors, substantial amounts of Ras proteins have also been identified in subcellular organelles where they can elicit several functions. Over the last decades, the combination of cellular fractionation techniques with newly developed advanced microscopy revealed the presence of Ras proteins in endosomal fractions, visualized their trafficking to endosomal structures in live cells, and provided extensive colocalization data with established markers of the different endocytic compartments (Lu et al. 2009; Moreto et al. 2008, 2009; Pol et al. 1998; Howe et al. 2001; Fivaz and Meyer 2005; Jiang and Sorkin 2002; Gomez and Daniotti 2007; Roy et al. 2002; Yeung et al. 2008; Hancock 2003; Gelabert-Baldrich et al. 2014; Choy et al. 1999; Zheng et al. 2012b; Prior and Hancock 2012). Most of the results have been obtained with ectopic expression of fluorescently tagged wildtype, active and inactive Ras mutant proteins in different cell types. Using advanced imaging like FRET, FRAP technology or in the case of KRas, RAICHU probes, these fusion constructs were critical tools to develop experimental approaches that were able to monitor the location and activity of Ras proteins over time in live and fixed cells. As the availability of sensitive antibodies specific for Ras isoforms and their activity remains an issue in the field, these fluorescently labeled Ras proteins have been proven very valuable. However, despite the great insights obtained with these methodologies, it should be noted that a lot of the conclusions drawn from these overexpression studies still lack validation for their endogenous counterparts.

In general, Ras proteins can be translocated from the plasma membrane to endosomes via two different routes. The pathway mainly used by H-, N-, and KRas4A is through endocytic vesicles derived from CDE and CIE pathways (Porat-Shliom et al. 2008; Howe et al. 2001; Gomez and Daniotti 2005; Jiang and Sorkin 2002; Roy et al. 2002) (see [6] in Fig. 3.1). The other route predominantly used by KRas involves desorption from the plasma membrane into the cytosol, followed by non-vesicular diffusion shuttling mechanism to endosomes (Gelabert-Baldrich et al. 2014; Fivaz and Meyer 2005; Yeung et al. 2008; Schmick et al. 2015) (see [5] in Fig. 3.1).

Endocytosed palmitoylated Ras proteins, via CDE or Arf6-dependent CIE encounter the EE or RE compartment in a process regulated by Rab5 or Rab11 GTPases (Gomez and Daniotti 2005; Porat-Shliom et al. 2008; Howe et al. 2001) (see [6] in Fig. 3.1). In EEs, H- and NRas can be mono- and di-ubiquitinated via

a lysine 63-linked chains on lysine 117, lysine 147, and lysine 170 independently of their activation state (Jura et al. 2006). This is not a degradative posttranslational modification but rather stabilizes these Ras isoforms in endosomes and inhibits their recycling to plasma membrane (Jura et al. 2006) (see [7] in Fig. 3.1). The HVR of these Ras proteins is not an obligatory acceptor for ubiquitin but participates in ubiquitination by the E3 ubiquitin ligase Rabex5 (Xu et al. 2010). Interestingly, activated Ras induces the recruitment of Rabex-5 to endosomes through a RAS and RAB interactor 1 (RIN1)-containing machinery. Consequently, this leads to Rab5 activation. Hence, through this complex interplay of Ras with ubiquitination and Rab5 activity, Ras modulates its own stability in endosomes (Zheng et al. 2012b; Ahearn et al. 2011). In addition, retention of Ras proteins in endosomes is also regulated by several members of the sorting nexin (SNX) family such as SNX17, SNX27, and SNX31, which bind PtdIns(3)P as well as active Ras on endosomes (Ghai et al. 2011).

In contrast to H- and NRas, KRas is mono-ubiquitinated via lysine 45-linked chain on lysine 104 and lysine 147 and this modification does not affect its endosomal trafficking but enhances interactions with several effectors (Sasaki et al. 2011). Overall, the endosomal localization of KRas is less prominent compared to H- and NRas isoforms, probably because KRas is less retained on endosomes and as a result faster recycling to the plasma membrane may occur (Vigil et al. 2010; Jiang and Sorkin 2002; Roy et al. 2002). Yet, despite the small amount of KRas in EE, KRas can also continue its journey to LE/MVBs and eventually into lysosomes following the degradative endocytic pathway (Lu et al. 2009) (see [8] in Fig. 3.1). This feature is strikingly different to H- and NRas, which are essentially found in EEs and REs following the recycling route to the plasma membrane (see [9] in Fig. 3.1). The trafficking of GFP-tagged KRas through these different compartments was confirmed by colocalization with established endocytic markers for EE, LE and LE/lysosomes (Lu et al. 2009). Moreover, subcellular fractionation identified approximately 10% of ectopically expressed GFP-KRas, but also endogenous KRas independent of its activation state, in purified EE and LE fractions (Lu et al. 2009; Gelabert-Baldrich et al. 2014). The trafficking routes that deliver KRas to LE and lysosomes are not fully resolved. As GFP-KRas is observed in clathrin-coated pits and vesicles, KRas may reach EE/LE/MVB/lysosomes via CDE and endosomal transport vesicles (Lu et al. 2009). However, FRAP microscopy implicated that delivery through transport vesicles accounted for only a minor proportion of GFP-KRas on endosomal membranes (Gelabert-Baldrich et al. 2014).

FRAP analysis identified a fast replenishment (half-time 1.3 s) of approximately 80% of the bleached GFP-KRas pool on endosomes. This indicates that KRas is highly dynamic, which is in agreement with a model of rapid diffusional incorporation from the cytoplasm. Strikingly different from KRas, the fluorescence recovery was negligible for GFP-H- and GFP-KRas4A, strongly supporting their dependence in slow vesicular transport (Gelabert-Baldrich et al. 2014).

Based on these findings, one can assume that cellular stimuli that lead to KRas serine 181 phosphorylation or promote association of known PBR/farnesyl-binding proteins to the active KRas (CaM, PRA1, PDE-δ) could regulate KRas interaction dynamics with endosomes by inducing electrostatic switch or membrane dissocia-

tion, respectively (Alvarez-Moya et al. 2011; Bhagatji et al. 2010; Fivaz and Meyer 2005; Lopez-Alcala et al. 2008; Chandra et al. 2011; Bivona et al. 2006). Indeed, FRAP microscopy in COS1 cells identified that the constitutively inactive KRas mutant KRasS17N was more immobile than the active mutant KRasG12V as a consequence that active KRas, but not the inactive, can bind CaM or can be posttranslationally modified via serine 181 phosphorylation (Gelabert-Baldrich et al. 2014) (see [5] Fig. 3.1).

As previously mentioned, the PDE-δ protein, through its interaction with the farnesyl group of KRas, regulates KRas localization and dynamics on endosomal membranes and plasma membrane. This interaction solubilizes KRas from endomembranes with low negatively charged surfaces, including EE membranes, leading to a redistribution of KRas to perinuclear RE membranes. The underlying mechanism for this redistribution was based on an activity in RE that displaced KRas from PDE-δ. Further studies then identified the Arf-like GTPase Arl2, which in its active form binds to an allosteric site on PDE-δ, thereby inducing a conformational change that unloads farnesylated cargo (Ismail et al. 2011). Finally, from RE, KRas follows the recycling pathway to the plasma membrane through vesicular transport (Schmick et al. 2015) (see [10] in Fig. 3.1). Trafficking of the different isoforms is summarized in Fig. 3.1.

3.2.1.4 Ras Signaling Pathways from Endosomes

Several studies identified upstream components of the Ras activation pathway on endosomes, including activated EGFR and adaptor proteins Shc/Grb2. This complex is able to recruit Sos1, facilitating GDP/GTP exchange for increasing Ras activity in the endosomal compartment (von Zastrow and Sorkin 2007; Vieira et al. 1996; Herbst et al. 1994; Jiang and Sorkin 2002; Wang et al. 2002). Ras activation on endosomes in live cells has been elegantly demonstrated using FRET microscopy, which allows the spatiotemporal analysis of interaction between molecules inside cells (Jiang and Sorkin 2002; Moreto et al. 2008; Miaczynska and Bar-Sagi 2010). This methodology is based on the energy transfer between two spectrally overlapping GFP variants in cells, for instance, Cyan/cerulean-FP acting as a donor and Yellow/Venus-FP as an acceptor of energy. In order to determine Ras activation utilizing FRET, one of the GFP variants were fused with Ras, while the other GFP variants was fused with the Ras-binding domain of the Raf1 effector. This approach appeared appropriate to detect Ras activation on endosomes (Lu et al. 2009; Jiang and Sorkin 2002; Gomez and Daniotti 2005; Misaki et al. 2010), in particular, as phosphorylated and activated Raf1 was present in purified endosomes from rat liver (Di Guglielmo et al. 1994; Pol et al. 1998). In addition, bimolecular fluorescent complementation (BiFC) microscopy has been also very useful to characterize several other Ras effectors that interact directly or in a protein complex with active Ras on endosomes, such as PI3K and Cdc42 (Tsutsumi et al. 2009; Chang and Philips 2006; Cheng et al. 2011). The BiFC technique relies on the fusion of the N- and C-terminal Venus fluorescent protein with Ras and the RBD of the selected effector,

respectively. Once both proteins interact, the Venus fluorophore rebuilds by complementation, generating a fluorescence signal that can be monitored using microscopy (Zheng and Chang 2014).

The FRET technology described above is based on the overexpression of two fluorescent proteins, often creating false FRET signals that did not correlate with endogenous Ras activation. Unimolecular FRET technology using Raichu probes provided a great advancement for the field. In this approach, YFP-KRas is fused to the CFP-tagged RBD of Raf-1 to provide a single ('biosensor') construct that upon KRas activation, allows intramolecular binding to Raf-RBD. This ultimately brought CFP and YFP in close proximity to create a detectable FRET signal. Using this Raichu probe, it has been demonstrated that EGF-induced activation of KRas in live cells (Lu et al. 2009; Kiyokawa et al. 2006; Mochizuki et al. 2001; Miaczynska and Bar-Sagi 2010).

Despite these findings, the general perception in the field still considers Ras activation at the plasma membrane rather than endosomal signaling as the driver of oncogenic events. However, in NIH3T3 cells, inhibition of Ras internalization to exclusively examine Ras signaling emanating from the cell surface was inefficient to effectively induce cell transformation (Cheng et al. 2011). In addition, a focus formation assay identified expression of endosomal GFP-Ras to produce more foci than the GFP transfected control (Aran and Prior 2013). Hence, endosomal Ras signaling seems to substantially contribute to oncogenic events. In the following, we will dissect some of the Ras isoforms and their effector pathways and endosomal locations that possibly contribute to cell transformation.

The identification of signal specificity within the Ras family has captivated the field for a long time, as the highly conserved effector binding G-domain in all active Ras (GTP-bound) isoforms is capable to interact with the same set of effectors (Wittinghofer and Herrmann 1995) to potentially elicit the same signaling output. Out of more than 20 known Ras effectors, Raf1 and PI3K have been the most extensively studied, driving MEK/MAPK and Akt signaling cascades that control proliferation and cell survival, respectively. Based on their similar binding behavior, it was initially proposed that Ras isoforms would have no preference to couple with either Raf1/MAPK or PI3K/Akt (Omerovic et al. 2008). However, more recent work revealed that depending on the activated Ras isoform, Raf1/MAPK and PI3K/Akt effector pathways appear to be differentially regulated on endosomes. This observation seems to be a consequence of the Ras isoform-specific posttranslational ubiquitin modifications discussed previously. The ubiquitination of H- and NRas on endosomes, which stabilizes their association with EE and simultaneously, reduces recycling to the plasma membrane (see Sect. 3.2.1.3), impairs interaction of H- and NRas with Raf1. Consequently, MAPK activation is reduced, yet PI3K/Akt activation is not affected (Xu et al. 2010; Yan et al. 2010; Jura et al. 2006). Hence, inhibition or overexpression of the ubiquitin ligase Rabex5, which is responsible for H- and NRas ubiquitination on endosomes, resulted in increased or attenuated Raf1/MAPK activation, respectively (Xu et al. 2010; Yan et al. 2010). In a much more complex scenario, Rin1, the GEF for Rab5, can bind HRas on endosomes and simultaneously stimulates Rab5-dependent endocytosis (Cheng et al. 2011; Tall et al. 2001). If the

latter then triggers Rabex5 recruitment, this could also lead to HRas ubiquitination, thereby generating a negative feedback mechanism for HRas/MAPK activation.

Despite the substantial amount of data generated from multiple research groups in recent years on Ras signaling from endosomes, including FRET microscopy demonstrating interaction of ectopically expressed HRas with Raf1 on endosomes (Jiang and Sorkin 2002), Sorkin and coworkers recently reported that endogenous HRas signals from receptors activated at the plasma membrane and not from internal membranes (Pinilla-Macua et al. 2016). These findings clearly challenge the now widely accepted concept of HRas-mediated Raf1/MAPK activation on endosomes highlighting the need for cautious interpretation of data based on ectopic Ras overexpression, which generate highly elevated Ras levels compared to their endogenous counterpart. Hence, future research with advanced technology that would clarify the signaling activities of endogenous HRas on endosomes is still required.

In contrast to the inhibitory impact of ubiquitination on H- and NRas activity, the same posttranslational modification enhances KRas activity. This increases interaction with Raf1 and PI3K, thus elevating signal output of MAPK and Akt pathways (Sasaki et al. 2011). Interestingly, HRas- and KRas-mediated activation of the Raf1/MAPK cascade varies in its dependence on endocytosis, CaM and PI3K activity (Roy et al. 2002; Moreto et al. 2008, 2009). Actually, inhibition of CaM and/or PI3K impairs recycling from EEs, which seems to be linked to HRas/MAPK inhibition (Roy et al. 2002). In fact, CaM inhibition generates enlarged endosomes by preventing the exit of endocytosed molecules, such as the EGFR, from EEs (Tebar et al. 2002) by a molecular mechanism that involves PKC-δ activity (Llado et al. 2004) and actin polymerization (Llado et al. 2008).

In COS1 cells, inhibition of endocytosis via overexpression of the dominant-negative dynamin mutant dynK44A, negatively affects HRas-dependent activation of Raf1 (Moreto et al. 2008). Although dynK44A may also affect other cellular processes, Omevoric et al. also demonstrated that inhibition of receptor internalization reduced H- and N-, but not KRas-mediated Raf1 activation (Omerovic et al. 2008). Together with reports demonstrating HRas and Raf1 interaction in Rab11-positive recycling endosomes after serum stimulation (Gomez and Daniotti 2005), this may suggest that Raf1 activation by HRas indeed occurs in endosomes. Taken together, current views favor divergent roles of endosomes in H/NRas-mediated Raf1/MAPK activation. Signal outcome, either abrogation or stimulation, could be explained by the existence of two different pools of H/NRas on endosomes: ubiquitinated Ras (Raf1/MAPK incompetent) and non-ubiquitinated Ras (Raf1/MAPK competent). The final signal output from H- and NRas on endosomes would then depend on the balance between these two Ras pools, which could be modulated differently by environmental signals, experimental settings, and the cell type.

Besides the differential regulation of Ras isoforms in EE described above, an additional layer of signal specificity is achieved through the localization of KRas, but not H- and NRas in LE/MVBs/lysosomes. In COS1 cells, Raichu probes revealed that EGF stimulation increased KRas activity in LE/MVBs, which in combination with Raf1 and the p14/MP1 scaffold for MEK and ERK (Teis et al. 2002), activates MAPK in this compartment (Lu et al. 2009).

Most interestingly, although the pool of ubiquitinated H- and NRas displays impaired MAPK activation in EEs, these isoforms are perfectly competent to activate the Rho-GTPase Cdc42, thus regulating Cdc42-dependent cytoskeletal rearrangements and cell transformation when they are endocytosed (Cheng et al. 2011; Cheng and Chang 2011). These findings complement Ras function in the fission Yeast pombe, which only express one Ras protein, Ras1. At the plasma membrane, Ras1 activates Byr2 (a MEKK homolog)/MAPK but on endomembranes, Ras1 activates Sed1, a GEF for Cdc42 but not MAPK. Thus, in endosomes Ras also activates the cytoskeleton to maintain an elongated morphology, cell polarity, and mitosis (Chang and Philips 2006; Onken et al. 2006). Strikingly, HRas-mediated Cdc42 activation also seems relevant in vivo, as expression of a constitutively active HRas mutant restricted to endomembranes induced tumors in nude mice by a mechanism comprising HRas/Cdc42 complex formation (Cheng et al. 2011). This interaction may be mediated by Dbl, one of several GEFs for Cdc42 (Cheng et al. 2011; Cerione 2004). Moreover, the presence of NRas-, and to a minor extent, KRas-containing Cdc42 complexes in these studies could indicate their contribution to tumor initiation (Cheng et al. 2011).

That Ras isoform signaling from endosomal compartments is intimately linked to the endocytic machinery is further highlighted by a screen for HRas effectors in endosomes using BiFC methodology. This study confirmed interaction of ubiquitinated HRas with Cdc42 and PI3K in this compartment (Zheng and Chang 2014; Tsutsumi et al. 2009), but also identified CHMP6/VPS20 and VPS4A as HRas interaction partners, all proteins of the ESCRT-III complex that controls recycling from endosomes to the plasma membrane (Zheng et al. 2012a). One could speculate that the latter interaction could increase recycling of HRas, but also other components of endosomes, such as growth factor receptors, to enhance and sustain growth factor signaling.

Finally, among the different Ras effectors, it is noteworthy to mention T lymphoma invasion and metastasis-inducing 1 (Tiam1) (Lambert et al. 2002), which is a specific GEF for the small GTPase Rac1 (Habets et al. 1994). Interestingly, endosomal Tiam1 activates Rac1 in this compartment, a prerequisite for the subsequent transport of active Rac1 to specific plasma membrane domains and the generation of lamellipodia in migrating cells (Palamidessi et al. 2008). Together with Tiam1 being required for the development of Ras-induced skin tumors (Malliri et al. 2002), this points at Tiam1 as a critical link between Ras and Rac1 in metastasis. The role of Rac1 on endosomes will be explained in more detail in the following section. Figure 3.2 summarizes endosome signaling outcome of different Ras isoforms.

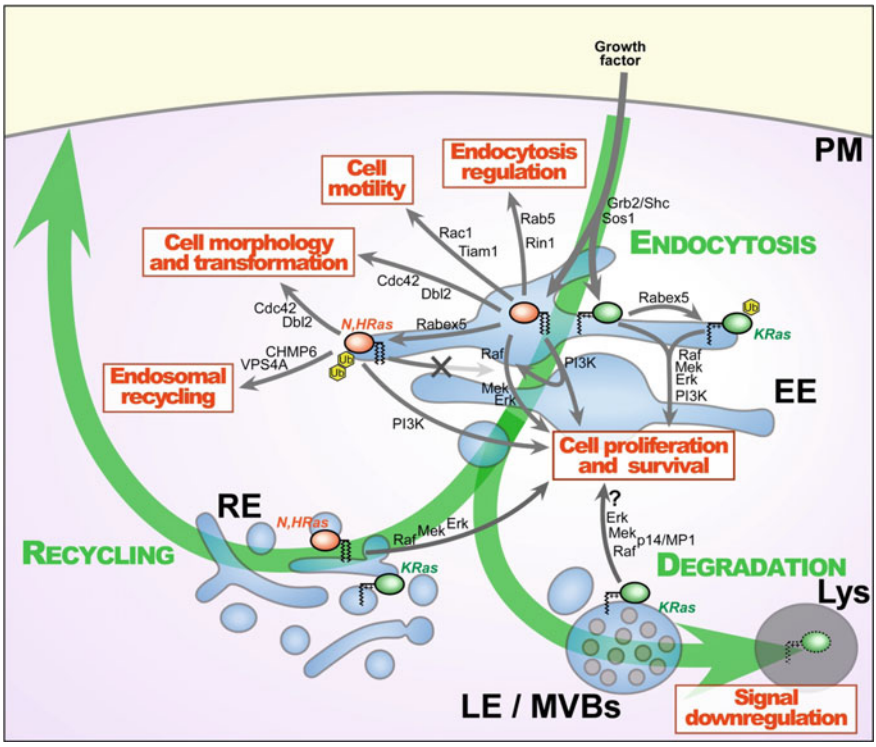

Fig. 3.2 Signaling pathways and outputs from endosomes generated by Ras GTPases. Growth factors induce Ras activation in early endosomes (EE) by recruiting the molecular machinery required for Ras-GTP loading, including adaptors Grb2, Shc, and the guanine exchange factor Sos1. In EE, activated H- or NRas (orange) and KRas (green) then signal through Raf1/Mek/Erk and PI3K/Akt pathways to regulate cell proliferation and survival, respectively. In the case of KRas, Erk activation is also elicited in late endosome/multivesicular bodies (LE/MVBs) through the Raf1/p14/MP1/Mek signaling cascade, which may also support cell proliferation. Furthermore, trafficking of KRas along the degradative route via LE/MVBs and lysosomes (Lys) finally downregulates KRas signaling. In addition, once in EEs, Ras isoforms then also follow the recycling pathway back to the plasma membrane (PM). Along this route, activation of the Raf1/Mek/Erk cascade can occur in the recycling endosomal compartment (RE), which consequently impacts on cell proliferation. In the EE compartment, Ras regulates several cellular processes by activating GEFs of other signaling proteins. In particular, Ras stimulates Tiam1-mediated Rac1 activation for cell motility and Rin1-mediated Rab5 activation to increase endocytosis. In EEs, Ras isoforms could also become substrates for the ubiquitin ligase Rabex5 for ubiquitination. This posttranslational modification enables all Ras isoforms to enhance PI3K activation but differentially affects the ability of H-, N-, or KRas to regulate Raf1 activity. On the other hand, ubiquitinated H- and NRas, through CHMP6 and VPS4A, increase overall transport through the recycling pathway. Additionally, both ubiquitinated as well as non-ubiquitinated H- and NRas activate Cdc42, through the GEF Dbl2, which may control cell morphology and transformation

3.2.2 *Rac1 Signaling from Endosomes*

Rac proteins belong to the Rho subfamily within the Ras superfamily of small GTPases (Bishop and Hall 2000; Didsbury et al. 1989). Mammalian organisms express three Rac isoforms: Rac1, the focus of this chapter, is ubiquitously expressed, while Rac2 is mostly expressed in the hematopoietic lineage and Rac3 is only found in the central nervous system (Didsbury et al. 1989; Bolis et al. 2003). Together with Rho and Cdc42, Rac1 represents one of the three most extensively studied Rho family members, all well known to coordinate cytoskeleton dynamics that control the formation of stress fibers, filopodia, and lamellipodia, respectively, for cell mobility (Bishop and Hall 2000; Hall 1998; Ridley 2001).

By controlling the dynamics of the actin cytoskeleton, Rac1 regulates many cellular processes linked to cell motility, including cell spreading, adhesion, migration, and axonal growth, but also phagocytosis, macropinocytosis, pinocytosis or vesicular transport (Bosco et al. 2009; Ridley 2006; Heasman and Ridley 2008; Bustelo et al. 2007). Moreover, a wide range of cellular functions, not strictly related with the control of the cytoskeleton, have also been described for Rac1, such as cell survival, the control of gene expression, cell cycle, cell differentiation as well as proliferation (Bosco et al. 2009; Sahai and Marshall 2002; Bishop and Hall 2000). In this context, some mitogenic Rac1 activities can be attributed to signaling events downstream of oncogenic Ras (Samuel et al. 2011; Joneson et al. 1996; Qiu et al. 1995).

Hence, given the multiple functions of Rac1 in fundamental cellular processes, deregulation of Rac1 has been identified to significantly contribute to pathogenic events in several human diseases, including cardiovascular diseases, and metastatic dissemination during cancer progression (Marei and Malliri 2016; Sahai and Marshall 2002). Indeed, Rac1 is a potent regulator of epithelial-mesenchymal transition (EMT) and its reverse process, mesenchymal-epithelial transition (MET), both considered critical to guide cell migration and metastasis of epithelial tumors (Marei and Malliri 2016).

Although activating mutations of Rac1 have only been found with a very low frequency in lung, skin or breast cancer (Schnelzer et al. 2000; Davis et al. 2013), its significant contribution to cell motility, metastasis and cancer progression has been thoroughly demonstrated (Parri and Chiarugi 2010; Bosco et al. 2009; Marei and Malliri 2016). However, despite these pro-oncogenic activities, Rac1 and its GEF Tiam1 also protect against invasion by stabilizing cadherin-mediated cell—cell contacts (Marei and Malliri 2016), and restoring epithelial morphology in Ras-transformed Madin Darby canine kidney cells (Hordijk et al. 1997). It is believed that these pro- and anti-invasive Rac1 activities could be triggered by different stimuli, or response to changes in the surrounding microenvironment, such as cell interaction with the ECM or reflect differential effects of Rac1 activity in relation to the stage of tumor progression (Sander et al. 1998; Sahai and Marshall 2002; De Franceschi et al. 2015; Marei and Malliri 2016; Bosco et al. 2009).

3.2.2.1 Synthesis, Processing and Trafficking of Rac1

In the cytosol, Rac1 is synthesized as a hydrophilic protein and like the Ras isoforms, contains a C-terminal CAAX motif (CLLL). The final leucine residue allows its recognition by a geranylgeranyltransferase type I that covalently incorporates a geranylgeranyl group (20-carbons isoprenyl) to the cysteine residue of the CLLL recognition sequence (Reid et al. 2004). This first cytosolic posttranslational modification enables incorporation of Rac1 into ER membranes and the subsequent AAX hydrolysis, followed by methylation of the geranylgeranylated cysteine residue mediated by Rce1 and Icmt enzymes, respectively, similar to the processing of Ras isoforms described above (Sect. 3.2.1.2). Adding additional complexity in the regulation of Rac1 processing, localization and activity, interaction with Rho-GDP dissociation inhibitor (RhoGDI) proteins then facilitates solubilization of ER-associated Rac1 and consequently, Rac1 release into the cytosol (Bustelo et al. 2007; Hoffman et al. 2000; Marei and Malliri 2016).

Like all other Ras GTPases, Rac1 switches between the active GTP-bound and inactive GDP-bound form, a cycle controlled by several Rho-GEFs and Rho-GAPs that ensure the spatiotemporal regulation of Rac1 activity (Sahai and Marshall 2002). However, in striking contrast to the Ras isoforms, the interaction of RhoGDIs with Rac1, as well as the other members of the Rho family, provides an additional layer to control Rac1 activity. RhoGDIs mainly interact with the inactive Rac1 (GDP-bound) via the G-domain and the geranylgeranyl group, which weakens the membrane anchoring provided by the geranylgeranyl group, thereby solubilizing and sequestering the inactive Rac1 GTPase into the cytosol (Olofsson 1999; DerMardirossian and Bokoch 2005; Grizot et al. 2001). Extensive research over the years identified multiple regulatory circuits driven by growth factors and other external stimuli that can modify this interaction. For instance, RhoGDI can be phosphorylated by PKC or p21-activated kinase (PAK), decreasing its affinity for Rac1-GDP and allowing the insertion of the Rac1 prenyl group into the plasma membrane, followed by GEF-mediated activation of Rac1 (DerMardirossian et al. 2004; Price et al. 2003). Also, integrins can displace RhoGDIs by favoring Rac1 insertion into lipid rafts, specialized plasma membrane domains associated with Rac1 activation (del Pozo et al. 2000, 2002). This complex regulatory circuit is initiated by ECM proteins, fibronectins, first activating integrins in lipid rafts. This stimulates phospholipase D (PLD) and leads to the localized generation of PA in this membrane domain, which competes with RhoGDI for the binding to Rac1. Ultimately, this ensures the Rac1 prenyl group insertion into this membrane domain, which favors Rac1 activation and consequently cell spreading, lamellipodia and migration (Chae et al. 2008).

Within the hypervariable region and adjacent to the CAAX motif, Rac1 also harbors a polybasic region. Several reports demonstrate that this PBR electrostatically interacts with anionic membrane phospholipids, like PS, PtdIns(3,4,5)P_3, PtdIns(3,5)P_2, or PA, determining its localization in specific membrane domains under different physiological conditions (Michaelson et al. 2001; ten Klooster and Hordijk 2007; Finkielstein et al. 2006; Yeung et al. 2006; Chae et al. 2008). In addi-

tion, a proline-rich domain neighboring the PBR has been demonstrated to contribute to the targeting of Rac1 to cellular focal adhesions (ten Klooster et al. 2006).

Finally, Rac1 is also palmitoylated at the cysteine 178 amino acid, which greatly enhances Rac1 membrane stability and promotes its localization and functionality in cholesterol-rich plasma membrane domains (lipid rafts) (Navarro-Lerida et al. 2012). Similar to the Ras isoforms, at the plasma membrane Rac1 can be internalized through CIE transport vesicles reaching EEs and then LEs. The role of Rac1 endocytosis in signaling and its implication in the regulation of different cellular processes is detailed in the next section.

3.2.2.2 Signaling Pathways Regulated by Rac1 from Endosomes

As the genetic ablation of Rac1 in mice results in embryonic lethality (Sugihara et al. 1998) limited information of Rac1 function in vivo is available up to date, mostly relying on studies modulating Rac1 effector gene expression. Therefore, the current knowledge in the field is still based on the vast majority of Rac1 signaling studies performed in cell culture experiments using different cell types.

Signaling elicited by Rac1 effectors commonly are related to actin cytoskeleton rearrangements. One of the most extensively studied Rac1 effectors is PAK which phosphorylates LIM kinase and cortactin, among others, to coordinate actin polymerization (F-actin) at the plasma membrane in a multifactorial process that includes the actin-related protein-2/3 (Arp2/3) complex, Neural Wiskott–Aldrich syndrome protein (N-WASP)/*WASP-family verprolin-homologous* protein (WAVE), cofilin or dynamin proteins (Frost et al. 1998; Yang et al. 1998; Vidal et al. 2002; Webb et al. 2006; Sauvonnet et al. 2005; Schafer et al. 2002; Grassart et al. 2010). In addition, another Rac1 effector determining actin dynamics is phosphatidylinositol-4-phosphate 5-kinase (Tolias and Carpenter 2000; Tolias et al. 2000; Vidal-Quadras et al. 2011; Weernink et al. 2004; Chao et al. 2010), which upon activation leads to increased production of PtdIns(4,5)P_2 at the plasma membrane (Doughman et al. 2003; Tolias et al. 2000; van den Bout and Divecha 2009; Shibasaki et al. 1997). On the other hand, Rac1 interacts and activates phospholipase C enzymes (Illenberger et al. 1998; Jezyk et al. 2006; Li et al. 2009), which hydrolyzes PtdIns(4,5)P_2 to generate diacylglycerol and PtdIns(3)P both well established second messengers. This rapid turnover of PtdIns(4,5)P_2 has been demonstrated to promote F-actin polymerization and cell migration (Li et al. 2009). The diversity and complexity of PIs modulating Rac1 signaling are further underscored with PI3K generating PtdIns(3,4,5)P_3 from PtdIns(4,5)P_2, which affects cell migration and cell survival controlled by Rac1 (Yang et al. 2011; Murga et al. 2002). Moreover, PtdIns(3,4,5)P_3 is able to recruit several Rac1-GEFs to the plasma membrane, providing multiple opportunities for positive feedback mechanisms between PI3K and Rac1 (Zhu et al. 2015; Ebi et al. 2013).

All of these Rac1 effectors are critical for actin organization at the plasma membrane, which in addition to controlling formation of membrane protrusions such as lamellipodia in migrating cells, also influences endocytosis (Lamaze et al. 1996;

Soriano-Castell et al. 2017). For instance, Rac1 impacts on CIE of receptors, such as IL-2R (Grassart et al. 2008; Lamaze et al. 2001), fluid phase ingestion, TrkA receptor internalization via macropinocytosis (Valdez et al. 2007), as well as phagocytosis of pathogens (Etienne-Manneville and Hall 2002; Criss et al. 2001). Interestingly, the recently identified novel Rac1 effector amyotrophic lateral sclerosis 2 (ALS2) gene, which is vital for motor neurons, is a Rab5-GEF driving Rac1 activation for macropinocytosis and the subsequent fusion of macropinosomes with EEs (Kunita et al. 2007).

As outlined above, membrane recruitment of Rac1 effectors is intimately linked to cholesterol- and sphingolipid-enriched domains, but is also strongly influenced by the distribution of phosphorylated derivatives of PI. All of these membrane lipids localize to distinct membrane domains at the plasma membrane and in endosomal compartments, contributing to the recruitment of distinct effectors that not only establish signaling platforms, but also control membrane dynamics. Thus, Rac1 does not only control endocytic transport (see above), but vice versa endocytic trafficking is also instrumental in modulating Rac1 activity. This intermingled connection between endocytic trafficking and signal output provides opportunity for localized Rac1 signaling within the endocytic compartment and has been thoroughly demonstrated for ectopically expressed and fluorescently tagged Rac1, Raichu sensors, or photoactivatable GFP-Rac1 by means of biochemical and microscopy techniques. For instance, endocytosis of activated growth factor receptor enabled Rac1 activation in EEs and LEs, while RE ensured the translocation of active Rac1 to specific plasma membrane domains to control decisive events enabling forward movement, such as cell–cell contact, focal adhesion dynamics, assembly and disassembly of invadopodia or lamellipodia membrane protrusion at the leading edge (Menard et al. 2014; Revach et al. 2016; Garcia-Weber and Millan 2016; Stasyk and Huber 2016; Miaczynska and Bar-Sagi 2010; De Franceschi et al. 2015; Zhou et al. 2007; Harrington et al. 2011). Indeed, Kermorgant and coworkers demonstrated that the signaling output from endosomes of activated HGF-R (Met) not only enables Rac1 activation in this compartment, but subsequently ensures redirection of active (GTP-bound) Rac1 to the plasma membrane to control cell migration (Barrow-McGee and Kermorgant 2014). This implicates Rac1 signaling events from endosomes as well as from the plasma membrane to cooperatively determine the migratory behavior of cells. Indeed, PI3K activity and the Rac1-GEF Vav2 in perinuclear endosomes are required to sustain Rac1 signaling output to efficiently activate cell migration and invasion (Menard et al. 2014; Joffre et al. 2011). Most strikingly, constitutively active and oncogenic Met mutants (M1268T and D1246N), which accumulate in endosomal compartments, are characterized by enhanced endosomal Rac1 activity, reduced actin stress fibers, and increased cell migration, highlighting the significant contribution of endocytosis, and endosomal Rac1 signaling to tumor progression and metastatic events (Joffre et al. 2011; Barrow-McGee and Kermorgant 2014).

Further underscoring endocytic trafficking substantially contributing to Rac1 activation, after growth factor-induced activation of motogenic receptors such as HGF-R, Rab5 activity, and CDE is required for Rac1 activation on EEs through the Rho-GEF Tiam1. Moreover, the recycling of active endosomal Rac1 back to the plasma mem-

brane, via the small GTPase Arf6, triggered the formation of actin-based migratory protrusions. This endocytic trafficking route of active Rac1 through Rab5- and Arf6-positive compartments seems to contribute to cell motility in a variety of tumor cells (Palamidessi et al. 2008). In support of the latter, it has been described that active Arf6 induces Rac1 activation through endosomal trafficking (Donaldson et al. 2009).

In other cell types and settings, the initiation of Rac1 signaling from endosomes does not always require growth factor activation. For example, in endothelial cells ECM-bound β1-integrins recruit another member of the Ras superfamily, R-Ras, to nascent adhesions in lamellipodia, which promotes β1-integrin and R-Ras internalization by a Rab5-dependent pathway. Once endocytosed, R-Ras then activates Rac1 through Tiam1, followed by active Rac1 redirection to the plasma membrane to control cell adhesion and morphogenesis (Sandri et al. 2012). Similarly, TrkB receptor-mediated activation of PI3K and Tiam1/Rac in endosomes is essential to mediate the chemotactic response of the brain-derived neurotrophic factor (Zhou et al. 2007).

While the majority of Rac1 signaling events described above seem to occur predominantly in EE and RE compartments, Rac1 activity has also been associated with proteins and events located in LE. Along this line, the small GTPase Rab7, an established LE marker that controls late endocytic trafficking, directly interacts with Rac1 and both proteins colocalize in endosomes at the perinuclear region and on vesicles near the plasma membrane. It has been recently demonstrated that Rab7 enables Rac1 activation and promotes Rac1 delivery to the plasma membrane to stimulate cell migration (Margiotta et al. 2017). Moreover, Rab7 and Rac1 association also facilitates endosomal transport, through microtubules and actin filaments, in the context of ruffled border formation in osteoclasts, E-cadherin turnover, and stability of cell-cell contacts (Sun et al. 2005; Frasa et al. 2010).

In the LE compartment, the small GTPase RhoB acts as a negative regulator of Rac1 activity. Hence, inhibition of RhoB induced Rac1 activity and consequently lamellipodia protrusion (Garcia-Weber and Millan 2016). In contrast, active RhoB retained Rac1 in intracellular endosomal localizations and prevented Rac1 activation and its recycling to the cell border, blocking Rac1-dependent endothelial barrier reformation and stabilization of cell-cell junctions (Marcos-Ramiro et al. 2016). These findings are in consonance with the protective effect of HGF-induced and Tiam1-dependent Rac1 activation on endothelial cell barrier function, also requiring the Rac1 effector cortactin and a formation of a cortical actin ring (Birukova et al. 2007). Other negative Rac1 regulators include Rab11-family interacting protein 3 (FIP3). In T-cells, this interaction diverts Rac1 to a Rab11-positive recycling perinuclear endosomes, restricting access of Rac1 to the plasma membrane. In fact, FIP3 silencing induced T-cell spreading, a process that is controlled by Rac1, suggesting endosomal trafficking of Rac1 to regulate T-cell spreading in the immunological synapse (Bouchet et al. 2016).

Finally, it should be noted that the localization of intracellular Rac1 regulators may include players not restricted to the EE, RE, and LE compartments. This includes Rab8, which has been implicated in exocytic/recycling membrane trafficking, but also actin and microtubule cytoskeletal rearrangements (Hattula et al. 2006; Huber

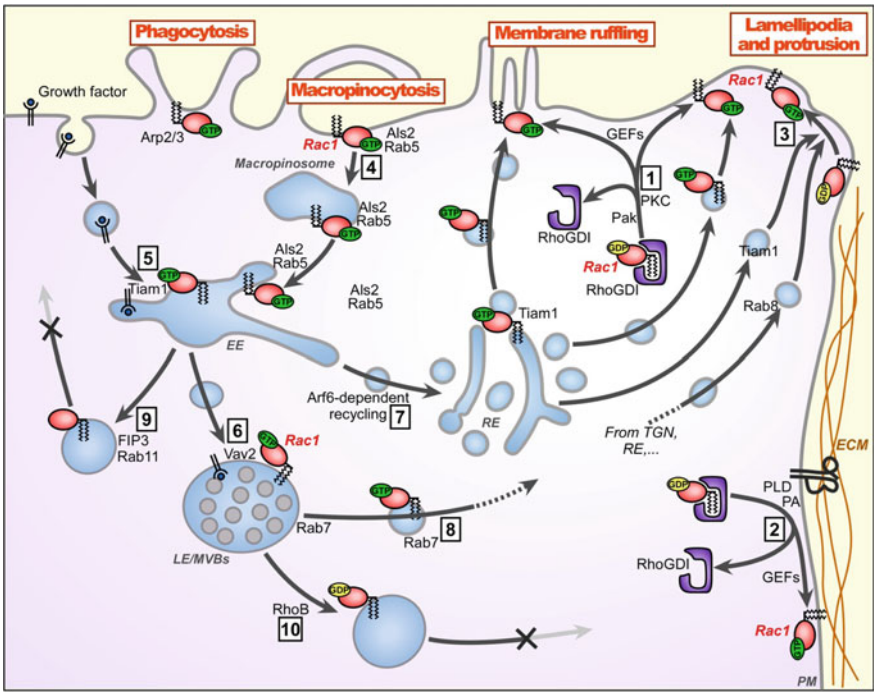

Fig. 3.3 Involvement of the endosomal compartment in Rac1 activation and trafficking to plasma membrane. This scheme illustrates the current knowledge on Rac1 activation on endosomes and Rac1 transport to the plasma membrane (PM) to regulate actin dynamics. In the cytosol, inactive Rac1 (GDP-bound, yellow) is sequestered and maintained soluble by RhoGDI. RhoGDI could be released by phosphorylation through activated p21-activated kinase (PAK)/protein kinase C (PKC) [1]. Alternatively, RhoGDI can be released by activated integrins bound to the extracellular matrix (ECM) upon phospholipase D (PLD) activation and generation of phosphatidic acid (PA), which competes with RhoGDI for Rac1 binding [2]. This allows the insertion of Rac1 at the PM and its interaction with GEFs, which are recruited to the PM by vesicular transport [3] or by interaction with elevated phosphoinositides at the PM. This interaction consequently leads to Rac1 activation (GTP-bound, green). At the PM, active Rac1 regulates cytoskeleton dynamics which controls phagocytosis, macropinocytosis, membrane ruffling, protrusion and lamellipodia formation. Exemplifying the multiple links between Rac1 activity and endocytosis, the Rac1-effector Als2 is a Rab5-GEF, which together with Rac1-induced actin rearrangements, activates macropinocytosis and the subsequent fusion of macropinosomes with early endosomes (EE) [4]. Endocytic membrane compartments and vesicular transport are also critical for Rac1 activation and its redirection to specific PM domains to exert the above-mentioned functions. Growth factor stimulation induces Rac1 activation in EEs and late endosomes (LEs/MVBs) by the GEFs Tiam1 [5] and Vav2 [6], respectively. Thereafter, recycling from these compartments by Arf6 [7], via the recycling compartment (RE), and Rab7 [8] redirects active Rac1 to specific domains at the PM. In addition, the Rab11 effector FIP3 in EEs [9] and the GTPase RhoB in LE/MVBs [10] have also been reported to deliver Rac1 to associated sequestering endocytic vesicles, which inactivate Rac1 by impairing its recycling to the PM

et al. 1993; Peranen et al. 1996; Roland et al. 2007). Rab8 localizes to vesicular structures, including RE and LE, but also the Golgi region, and peripheral membrane ruffles, and increases Rac1 activity and Tiam1/Rac1 mobilization from intracellular compartments to cortical locations to maintain directionality of migrating cells by enabling focal adhesion turnover and actin polymerization (Bravo-Cordero et al. 2016).

In summary, the aforementioned results exemplify and highlight the role of the endocytic system to regulate spatiotemporal Rac1 functionality. Several of these results have been illustrated in Fig. 3.3.

References

Ahearn IM, Haigis K, Bar-Sagi D, Philips MR (2011) Regulating the regulator: post-translational modification of RAS. Nat Rev Mol Cell Biol 13(1):39–51. https://doi.org/10.1038/nrm3255

Alanko J, Ivaska J (2016) Endosomes: emerging platforms for integrin-mediated FAK signalling. Trends Cell Biol 26(6):391–398. https://doi.org/10.1016/j.tcb.2016.02.001

Alanko J, Mai A, Jacquemet G, Schauer K, Kaukonen R, Saari M, Goud B, Ivaska J (2015) Integrin endosomal signalling suppresses anoikis. Nat Cell Biol 17(11):1412–1421. https://doi.org/10.1038/ncb3250

Alvarez-Moya B, Barcelo C, Tebar F, Jaumot M, Agell N (2011) CaM interaction and Ser181 phosphorylation as new K-Ras signaling modulators. Small GTPases 2(2):99–103. https://doi.org/10.4161/sgtp.2.2.15555

Apolloni A, Prior IA, Lindsay M, Parton RG, Hancock JF (2000) H-ras but not K-ras traffics to the plasma membrane through the exocytic pathway. Mol Cell Biol 20(7):2475–2487

Aran V, Prior IA (2013) Compartmentalized Ras signaling differentially contributes to phenotypic outputs. Cell Signal 25(9):1748–1753. https://doi.org/10.1016/j.cellsig.2013.05.004

Ariotti N, Fernandez-Rojo MA, Zhou Y, Hill MM, Rodkey TL, Inder KL, Tanner LB, Wenk MR, Hancock JF, Parton RG (2014) Caveolae regulate the nanoscale organization of the plasma membrane to remotely control Ras signaling. J Cell Biol 204(5):777–792. https://doi.org/10.1083/jcb.201307055

Balbis A, Parmar A, Wang Y, Baquiran G, Posner BI (2007) Compartmentalization of signaling-competent epidermal growth factor receptors in endosomes. Endocrinology 148(6):2944–2954. https://doi.org/10.1210/en.2006-1674

Ballester R, Furth ME, Rosen OM (1987) Phorbol ester- and protein kinase C-mediated phosphorylation of the cellular Kirsten ras gene product. J Biol Chem 262(6):2688–2695

Barbacid M (1987) ras genes. Annu Rev Biochem 56:779–827. https://doi.org/10.1146/annurev.bi.56.070187.004023

Barrow-McGee R, Kermorgant S (2014) Met endosomal signalling: in the right place, at the right time. Int J Biochem Cell Biol 49:69–74. https://doi.org/10.1016/j.biocel.2014.01.009

Bergeron JJ, Posner BI, Josefsberg Z, Sikstrom R (1978) Intracellular polypeptide hormone receptors. The demonstration of specific binding sites for insulin and human growth hormone in Golgi fractions isolated from the liver of female rats. J Biol Chem 253(11):4058–4066

Bhagatji P, Leventis R, Rich R, Lin CJ, Silvius JR (2010) Multiple cellular proteins modulate the dynamics of K-ras association with the plasma membrane. Biophys J 99(10):3327–3335. https://doi.org/10.1016/j.bpj.2010.10.001

Birukova AA, Alekseeva E, Mikaelyan A, Birukov KG (2007) HGF attenuates thrombin-induced endothelial permeability by Tiam1-mediated activation of the Rac pathway and by Tiam1/Rac-dependent inhibition of the Rho pathway. Faseb J 21(11):2776–2786. https://doi.org/10.1096/fj.06-7660com

Bishop AL, Hall A (2000) Rho GTPases and their effector proteins. Biochem J 348(Pt 2):241–255
Bivona TG, Quatela SE, Bodemann BO, Ahearn IM, Soskis MJ, Mor A, Miura J, Wiener HH, Wright L, Saba SG, Yim D, Fein A, Perez de Castro I, Li C, Thompson CB, Cox AD, Philips MR (2006) PKC regulates a farnesyl-electrostatic switch on K-Ras that promotes its association with Bcl-XL on mitochondria and induces apoptosis. Mol Cell 21(4):481–493. https://doi.org/10.1016/j.molcel.2006.01.012
Bolis A, Corbetta S, Cioce A, de Curtis I (2003) Differential distribution of Rac1 and Rac3 GTPases in the developing mouse brain: implications for a role of Rac3 in Purkinje cell differentiation. Eur J Neurosci 18(9):2417–2424
Bonifacino JS, Hurley JH (2008) Retromer. Curr Opin Cell Biol 20(4):427–436. https://doi.org/10.1016/j.ceb.2008.03.009
Bonifacino JS, Rojas R (2006) Retrograde transport from endosomes to the trans-Golgi network. Nat Rev Mol Cell Biol 7(8):568–579. https://doi.org/10.1038/nrm1985
Bos JL (1989) ras oncogenes in human cancer: a review. Cancer Res 49(17):4682–4689
Bos JL, Rehmann H, Wittinghofer A (2007) GEFs and GAPs: critical elements in the control of small G proteins. Cell 129(5):865–877. https://doi.org/10.1016/j.cell.2007.05.018
Bosco EE, Mulloy JC, Zheng Y (2009) Rac1 GTPase: a "Rac" of all trades. Cell Mol Life Sci 66(3):370–374. https://doi.org/10.1007/s00018-008-8552-x
Bouchet J, Del Rio-Iniguez I, Lasserre R, Aguera-Gonzalez S, Cuche C, Danckaert A, McCaffrey MW, Di Bartolo V, Alcover A (2016) Rac1-Rab11-FIP3 regulatory hub coordinates vesicle traffic with actin remodeling and T-cell activation. EMBO J 35(11):1160–1174. https://doi.org/10.15252/embj.201593274
Bravo-Cordero JJ, Marrero-Diaz R, Megias D, Genis L, Garcia-Grande A, Garcia MA, Arroyo AG, Montoya MC (2007) MT1-MMP proinvasive activity is regulated by a novel Rab8-dependent exocytic pathway. EMBO J 26(6):1499–1510. https://doi.org/10.1038/sj.emboj.7601606
Bravo-Cordero JJ, Cordani M, Soriano SF, Diez B, Munoz-Agudo C, Casanova-Acebes M, Boullosa C, Guadamillas MC, Ezkurdia I, Gonzalez-Pisano D, Del Pozo MA, Montoya MC (2016) A novel high-content analysis tool reveals Rab8-driven cytoskeletal reorganization through Rho GTPases, calpain and MT1-MMP. J Cell Sci 129(8):1734–1749. https://doi.org/10.1242/jcs.174920
Bustelo XR, Sauzeau V, Berenjeno IM (2007) GTP-binding proteins of the Rho/Rac family: regulation, effectors and functions in vivo. BioEssays 29(4):356–370. https://doi.org/10.1002/bies.20558
Calvo F, Agudo-Ibanez L, Crespo P (2010) The Ras-ERK pathway: understanding site-specific signaling provides hope of new anti-tumor therapies. BioEssays 32(5):412–421. https://doi.org/10.1002/bies.200900155
Castro-Castro A, Marchesin V, Monteiro P, Lodillinsky C, Rosse C, Chavrier P (2016) Cellular and molecular mechanisms of MT1-MMP-dependent cancer cell invasion. Annu Rev Cell Dev Biol 32:555–576. https://doi.org/10.1146/annurev-cellbio-111315-125227
Cerione RA (2004) Cdc42: new roads to travel. Trends Cell Biol 14(3):127–132. https://doi.org/10.1016/j.tcb.2004.01.008
Chae YC, Kim JH, Kim KL, Kim HW, Lee HY, Heo WD, Meyer T, Suh PG, Ryu SH (2008) Phospholipase D activity regulates integrin-mediated cell spreading and migration by inducing GTP-Rac translocation to the plasma membrane. Mol Biol Cell 19(7):3111–3123. https://doi.org/10.1091/mbc.E07-04-0337
Chandra A, Grecco HE, Pisupati V, Perera D, Cassidy L, Skoulidis F, Ismail SA, Hedberg C, Hanzal-Bayer M, Venkitaraman AR, Wittinghofer A, Bastiaens PI (2011) The GDI-like solubilizing factor PDEdelta sustains the spatial organization and signalling of Ras family proteins. Nat Cell Biol 14(2):148–158. https://doi.org/10.1038/ncb2394
Chang EC, Philips MR (2006) Spatial segregation of Ras signaling: new evidence from fission yeast. Cell Cycle 5(17):1936–1939. https://doi.org/10.4161/cc.5.17.3187
Chao WT, Daquinag AC, Ashcroft F, Kunz J (2010) Type I PIPK-alpha regulates directed cell migration by modulating Rac1 plasma membrane targeting and activation. J Cell Biol 190(2):247–262. https://doi.org/10.1083/jcb.200911110

Cheng CM, Chang EC (2011) Busy traveling Ras. Cell Cycle 10(8):1180–1181. https://doi.org/10.4161/cc.10.8.15259

Cheng CM, Li H, Gasman S, Huang J, Schiff R, Chang EC (2011) Compartmentalized Ras proteins transform NIH 3T3 cells with different efficiencies. Mol Cell Biol 31(5):983–997. https://doi.org/10.1128/MCB.00137-10

Cho KJ, Park JH, Piggott AM, Salim AA, Gorfe AA, Parton RG, Capon RJ, Lacey E, Hancock JF (2012) Staurosporines disrupt phosphatidylserine trafficking and mislocalize Ras proteins. J Biol Chem 287(52):43573–43584. https://doi.org/10.1074/jbc.M112.424457

Choy E, Chiu VK, Silletti J, Feoktistov M, Morimoto T, Michaelson D, Ivanov IE, Philips MR (1999) Endomembrane trafficking of ras: the CAAX motif targets proteins to the ER and Golgi. Cell 98(1):69–80. https://doi.org/10.1016/S0092-8674(00)80607-8

Criss AK, Ahlgren DM, Jou TS, McCormick BA, Casanova JE (2001) The GTPase Rac1 selectively regulates Salmonella invasion at the apical plasma membrane of polarized epithelial cells. J Cell Sci 114(Pt 7):1331–1341

Davis MJ, Ha BH, Holman EC, Halaban R, Schlessinger J, Boggon TJ (2013) RAC1P29S is a spontaneously activating cancer-associated GTPase. Proc Natl Acad Sci U S A 110(3):912–917. https://doi.org/10.1073/pnas.1220895110

De Franceschi N, Hamidi H, Alanko J, Sahgal P, Ivaska J (2015) Integrin traffic—the update. J Cell Sci 128(5):839–852. https://doi.org/10.1242/jcs.161653

Debanne MT, Evans WH, Flint N, Regoeczi E (1982) Receptor-rich intracellular membrane vesicles transporting asialotransferrin and insulin in liver. Nature 298(5872):398–400

del Pozo MA, Price LS, Alderson NB, Ren XD, Schwartz MA (2000) Adhesion to the extracellular matrix regulates the coupling of the small GTPase Rac to its effector PAK. EMBO J 19(9):2008–2014. https://doi.org/10.1093/emboj/19.9.2008

del Pozo MA, Kiosses WB, Alderson NB, Meller N, Hahn KM, Schwartz MA (2002) Integrins regulate GTP-Rac localized effector interactions through dissociation of Rho-GDI. Nat Cell Biol 4(3):232–239. https://doi.org/10.1038/ncb759

DerMardirossian C, Bokoch GM (2005) GDIs: central regulatory molecules in Rho GTPase activation. Trends Cell Biol 15(7):356–363. https://doi.org/10.1016/j.tcb.2005.05.001

DerMardirossian C, Schnelzer A, Bokoch GM (2004) Phosphorylation of RhoGDI by Pak1 mediates dissociation of Rac GTPase. Mol Cell 15(1):117–127. https://doi.org/10.1016/j.molcel.2004.05.019

Di Guglielmo GM, Baass PC, Ou WJ, Posner BI, Bergeron JJ (1994) Compartmentalization of SHC, GRB2 and mSOS, and hyperphosphorylation of Raf-1 by EGF but not insulin in liver parenchyma. EMBO J 13(18):4269–4277

Di Guglielmo GM, Le Roy C, Goodfellow AF, Wrana JL (2003) Distinct endocytic pathways regulate TGF-beta receptor signalling and turnover. Nat Cell Biol 5(5):410–421. https://doi.org/10.1038/ncb975

Didsbury J, Weber RF, Bokoch GM, Evans T, Snyderman R (1989) rac, a novel ras-related family of proteins that are botulinum toxin substrates. J Biol Chem 264(28):16378–16382

Dobrowolski R, De Robertis EM (2011) Endocytic control of growth factor signalling: multivesicular bodies as signalling organelles. Nat Rev Mol Cell Biol 13(1):53–60. https://doi.org/10.1038/nrm3244

Donaldson JG, Porat-Shliom N, Cohen LA (2009) Clathrin-independent endocytosis: a unique platform for cell signaling and PM remodeling. Cell Signal 21(1):1–6. https://doi.org/10.1016/j.cellsig.2008.06.020

Doughman RL, Firestone AJ, Wojtasiak ML, Bunce MW, Anderson RA (2003) Membrane ruffling requires coordination between type Ialpha phosphatidylinositol phosphate kinase and Rac signaling. J Biol Chem 278(25):23036–23045. https://doi.org/10.1074/jbc.M211397200

Downward J (1996) Control of ras activation. Cancer Surv 27:87–100

Duan Y, Li F, Tan K, Liu H, Li Y, Liu Y, Kong X, Tang Y, Wu G, Yin Y (2015) Key mediators of intracellular amino acids signaling to mTORC1 activation. Amino Acids 47(5):857–867. https://doi.org/10.1007/s00726-015-1937-x

Ebi H, Costa C, Faber AC, Nishtala M, Kotani H, Juric D, Della Pelle P, Song Y, Yano S, Mino-Kenudson M, Benes CH, Engelman JA (2013) PI3K regulates MEK/ERK signaling in breast cancer via the Rac-GEF, P-Rex1. Proc Natl Acad Sci U S A 110(52):21124–21129. https://doi.org/10.1073/pnas.1314124110

Eisenberg S, Henis YI (2008) Interactions of Ras proteins with the plasma membrane and their roles in signaling. Cell Signal 20(1):31–39. https://doi.org/10.1016/j.cellsig.2007.07.012

Elad-Sfadia G, Haklai R, Balan E, Kloog Y (2004) Galectin-3 augments K-Ras activation and triggers a Ras signal that attenuates ERK but not phosphoinositide 3-kinase activity. J Biol Chem 279(33):34922–34930. https://doi.org/10.1074/jbc.M312697200

Etienne-Manneville S, Hall A (2002) Rho GTPases in cell biology. Nature 420(6916):629–635. https://doi.org/10.1038/nature01148

Fehrenbacher N, Bar-Sagi D, Philips M (2009) Ras/MAPK signaling from endomembranes. Mol Oncol 3(4):297–307. https://doi.org/10.1016/j.molonc.2009.06.004

Figueroa C, Taylor J, Vojtek AB (2001) Prenylated Rab acceptor protein is a receptor for prenylated small GTPases. J Biol Chem 276(30):28219–28225. https://doi.org/10.1074/jbc.M101763200

Finkielstein CV, Overduin M, Capelluto DG (2006) Cell migration and signaling specificity is determined by the phosphatidylserine recognition motif of Rac1. J Biol Chem 281(37):27317–27326. https://doi.org/10.1074/jbc.M605560200

Fivaz M, Meyer T (2005) Reversible intracellular translocation of KRas but not HRas in hippocampal neurons regulated by Ca^{2+}/calmodulin. J Cell Biol 170(3):429–441. https://doi.org/10.1083/jcb.200409157

Flinn RJ, Yan Y, Goswami S, Parker PJ, Backer JM (2010) The late endosome is essential for mTORC1 signaling. Mol Biol Cell 21(5):833–841. https://doi.org/10.1091/mbc.E09-09-0756

Fotiadou PP, Takahashi C, Rajabi HN, Ewen ME (2007) Wild-type NRas and KRas perform distinct functions during transformation. Mol Cell Biol 27(19):6742–6755. https://doi.org/10.1128/MCB.00234-07

Frasa MA, Maximiano FC, Smolarczyk K, Francis RE, Betson ME, Lozano E, Goldenring J, Seabra MC, Rak A, Ahmadian MR, Braga VM (2010) Armus is a Rac1 effector that inactivates Rab7 and regulates E-cadherin degradation. Curr Biol 20(3):198–208. https://doi.org/10.1016/j.cub.2009.12.053

Frittoli E, Palamidessi A, Disanza A, Scita G (2011) Secretory and endo/exocytic trafficking in invadopodia formation: the MT1-MMP paradigm. Eur J Cell Biol 90(2–3):108–114. https://doi.org/10.1016/j.ejcb.2010.04.007

Frost JA, Khokhlatchev A, Stippec S, White MA, Cobb MH (1998) Differential effects of PAK1-activating mutations reveal activity-dependent and -independent effects on cytoskeletal regulation. J Biol Chem 273(43):28191–28198

Garcia-Melero A, Reverter M, Hoque M, Meneses-Salas E, Koese M, Conway JR, Johnsen CH, Alvarez-Guaita A, Morales-Paytuvi F, Elmaghrabi YA, Pol A, Tebar F, Murray RZ, Timpson P, Enrich C, Grewal T, Rentero C (2016) Annexin A6 and late endosomal cholesterol modulate integrin recycling and cell migration. J Biol Chem 291(3):1320–1335. https://doi.org/10.1074/jbc.M115.683557

Garcia-Weber D, Millan J (2016) Parallels between single cell migration and barrier formation: the case of RhoB and Rac1 trafficking. Small GTPases 1–7. https://doi.org/10.1080/21541248.2016.1231655

Gelabert-Baldrich M, Soriano-Castell D, Calvo M, Lu A, Vina-Vilaseca A, Rentero C, Pol A, Grinstein S, Enrich C, Tebar F (2014) Dynamics of KRas on endosomes: involvement of acidic phospholipids in its association. Faseb J 28(7):3023–3037. https://doi.org/10.1096/fj.13-241158

Ghai R, Mobli M, Norwood SJ, Bugarcic A, Teasdale RD, King GF, Collins BM (2011) Phox homology band 4.1/ezrin/radixin/moesin-like proteins function as molecular scaffolds that interact with cargo receptors and Ras GTPases. Proc Natl Acad Sci U S A 108(19):7763–7768. https://doi.org/10.1073/pnas.1017110108

Gomez GA, Daniotti JL (2005) H-Ras dynamically interacts with recycling endosomes in CHO-K1 cells: involvement of Rab5 and Rab11 in the trafficking of H-Ras to this pericentriolar endocytic compartment. J Biol Chem 280(41):34997–35010. https://doi.org/10.1074/jbc.M506256200

Gomez GA, Daniotti JL (2007) Electrical properties of plasma membrane modulate subcellular distribution of K-Ras. FEBS J 274(9):2210–2228. https://doi.org/10.1111/j.1742-4658.2007.05758.x

Gonnord P, Blouin CM, Lamaze C (2012) Membrane trafficking and signaling: two sides of the same coin. Semin Cell Dev Biol 23(2):154–164. https://doi.org/10.1016/j.semcdb.2011.11.002

Goodwin JS, Drake KR, Rogers C, Wright L, Lippincott-Schwartz J, Philips MR, Kenworthy AK (2005) Depalmitoylated Ras traffics to and from the Golgi complex via a nonvesicular pathway. J Cell Biol 170(2):261–272. https://doi.org/10.1083/jcb.200502063

Gorfe AA, Hanzal-Bayer M, Abankwa D, Hancock JF, McCammon JA (2007) Structure and dynamics of the full-length lipid-modified H-Ras protein in a 1,2-dimyristoylglycero-3-phosphocholine bilayer. J Med Chem 50(4):674–684. https://doi.org/10.1021/jm061053f

Gould GW, Lippincott-Schwartz J (2009) New roles for endosomes: from vesicular carriers to multi-purpose platforms. Nat Rev Mol Cell Biol 10(4):287–292. https://doi.org/10.1038/nrm2652

Grassart A, Dujeancourt A, Lazarow PB, Dautry-Varsat A, Sauvonnet N (2008) Clathrin-independent endocytosis used by the IL-2 receptor is regulated by Rac1, Pak1 and Pak2. EMBO Rep 9(4):356–362. https://doi.org/10.1038/embor.2008.28

Grassart A, Meas-Yedid V, Dufour A, Olivo-Marin JC, Dautry-Varsat A, Sauvonnet N (2010) Pak1 phosphorylation enhances cortactin-N-WASP interaction in clathrin-caveolin-independent endocytosis. Traffic 11(8):1079–1091. https://doi.org/10.1111/j.1600-0854.2010.01075.x

Grewal T, Enrich C (2006) Molecular mechanisms involved in Ras inactivation: the annexin A6-p120GAP complex. BioEssays 28(12):1211–1220. https://doi.org/10.1002/bies.20503

Grizot S, Faure J, Fieschi F, Vignais PV, Dagher MC, Pebay-Peyroula E (2001) Crystal structure of the Rac1-RhoGDI complex involved in nadph oxidase activation. Biochemistry 40(34):10007–10013

Habets GG, Scholtes EH, Zuydgeest D, van der Kammen RA, Stam JC, Berns A, Collard JG (1994) Identification of an invasion-inducing gene, Tiam-1, that encodes a protein with homology to GDP-GTP exchangers for Rho-like proteins. Cell 77(4):537–549

Hall A (1998) Rho GTPases and the actin cytoskeleton. Science 279(5350):509–514

Hancock JF (2003) Ras proteins: different signals from different locations. Nat Rev Mol Cell Biol 4(5):373–384. https://doi.org/10.1038/nrm1105

Hancock JF, Magee AI, Childs JE, Marshall CJ (1989) All ras proteins are polyisoprenylated but only some are palmitoylated. Cell 57(7):1167–1177

Hancock JF, Paterson H, Marshall CJ (1990) A polybasic domain or palmitoylation is required in addition to the CAAX motif to localize p21ras to the plasma membrane. Cell 63(1):133–139

Harrington AW, St Hillaire C, Zweifel LS, Glebova NO, Philippidou P, Halegoua S, Ginty DD (2011) Recruitment of actin modifiers to TrkA endosomes governs retrograde NGF signaling and survival. Cell 146(3):421–434. https://doi.org/10.1016/j.cell.2011.07.008

Hattula K, Furuhjelm J, Tikkanen J, Tanhuanpaa K, Laakkonen P, Peranen J (2006) Characterization of the Rab8-specific membrane traffic route linked to protrusion formation. J Cell Sci 119(Pt 23):4866–4877. https://doi.org/10.1242/jcs.03275

Hayer A, Stoeber M, Ritz D, Engel S, Meyer HH, Helenius A (2010) Caveolin-1 is ubiquitinated and targeted to intralumenal vesicles in endolysosomes for degradation. J Cell Biol 191(3):615–629. https://doi.org/10.1083/jcb.201003086

Hayes S, Chawla A, Corvera S (2002) TGF beta receptor internalization into EEA1-enriched early endosomes: role in signaling to Smad2. J Cell Biol 158(7):1239–1249

Heasman SJ, Ridley AJ (2008) Mammalian Rho GTPases: new insights into their functions from in vivo studies. Nat Rev Mol Cell Biol 9(9):690–701. https://doi.org/10.1038/nrm2476

Heo WD, Inoue T, Park WS, Kim ML, Park BO, Wandless TJ, Meyer T (2006) PI(3,4,5)P_3 and PI(4,5)P_2 lipids target proteins with polybasic clusters to the plasma membrane. Science 314(5804):1458–1461. https://doi.org/10.1126/science.1134389

Herbst JJ, Opresko LK, Walsh BJ, Lauffenburger DA, Wiley HS (1994) Regulation of postendocytic trafficking of the epidermal growth factor receptor through endosomal retention. J Biol Chem 269(17):12865–12873

Hobbs GA, Der CJ, Rossman KL (2016) RAS isoforms and mutations in cancer at a glance. J Cell Sci 129(7):1287–1292. https://doi.org/10.1242/jcs.182873

Hoffman GR, Nassar N, Cerione RA (2000) Structure of the Rho family GTP-binding protein Cdc42 in complex with the multifunctional regulator RhoGDI. Cell 100(3):345–356

Hordijk PL, ten Klooster JP, van der Kammen RA, Michiels F, Oomen LC, Collard JG (1997) Inhibition of invasion of epithelial cells by Tiam1-Rac signaling. Science 278(5342):1464–1466

Howe CL, Valletta JS, Rusnak AS, Mobley WC (2001) NGF signaling from clathrin-coated vesicles: evidence that signaling endosomes serve as a platform for the Ras-MAPK pathway. Neuron 32(5):801–814

Huber LA, Pimplikar S, Parton RG, Virta H, Zerial M, Simons K (1993) Rab8, a small GTPase involved in vesicular traffic between the TGN and the basolateral plasma membrane. J Cell Biol 123(1):35–45

Huotari J, Helenius A (2011) Endosome maturation. EMBO J 30(17):3481–3500. https://doi.org/10.1038/emboj.2011.286

Illenberger D, Schwald F, Pimmer D, Binder W, Maier G, Dietrich A, Gierschik P (1998) Stimulation of phospholipase C-beta2 by the Rho GTPases Cdc42Hs and Rac1. EMBO J 17(21):6241–6249. https://doi.org/10.1093/emboj/17.21.6241

Ismail SA, Chen YX, Rusinova A, Chandra A, Bierbaum M, Gremer L, Triola G, Waldmann H, Bastiaens PI, Wittinghofer A (2011) Arl2-GTP and Arl3-GTP regulate a GDI-like transport system for farnesylated cargo. Nat Chem Biol 7(12):942–949. https://doi.org/10.1038/nchembio.686

Jezyk MR, Snyder JT, Gershberg S, Worthylake DK, Harden TK, Sondek J (2006) Crystal structure of Rac1 bound to its effector phospholipase C-beta2. Nat Struct Mol Biol 13(12):1135–1140. https://doi.org/10.1038/nsmb1175

Jiang X, Sorkin A (2002) Coordinated traffic of Grb2 and Ras during epidermal growth factor receptor endocytosis visualized in living cells. Mol Biol Cell 13(5):1522–1535. https://doi.org/10.1091/mbc.01-11-0552

Joffre C, Barrow R, Menard L, Calleja V, Hart IR, Kermorgant S (2011) A direct role for Met endocytosis in tumorigenesis. Nat Cell Biol 13(7):827–837. https://doi.org/10.1038/ncb2257

Johannes L, Popoff V (2008) Tracing the retrograde route in protein trafficking. Cell 135(7):1175–1187. https://doi.org/10.1016/j.cell.2008.12.009

Jones MC, Caswell PT, Norman JC (2006) Endocytic recycling pathways: emerging regulators of cell migration. Curr Opin Cell Biol 18(5):549–557. https://doi.org/10.1016/j.ceb.2006.08.003

Joneson T, White MA, Wigler MH, Bar-Sagi D (1996) Stimulation of membrane ruffling and MAP kinase activation by distinct effectors of RAS. Science 271(5250):810–812

Jovic M, Sharma M, Rahajeng J, Caplan S (2010) The early endosome: a busy sorting station for proteins at the crossroads. Histol Histopathol 25(1):99–112. https://doi.org/10.14670/HH-25.99

Jura N, Scotto-Lavino E, Sobczyk A, Bar-Sagi D (2006) Differential modification of Ras proteins by ubiquitination. Mol Cell 21(5):679–687. https://doi.org/10.1016/j.molcel.2006.02.011

Kajiho H, Kajiho Y, Frittoli E, Confalonieri S, Bertalot G, Viale G, Di Fiore PP, Oldani A, Garre M, Beznoussenko GV, Palamidessi A, Vecchi M, Chavrier P, Perez F, Scita G (2016) RAB2A controls MT1-MMP endocytic and E-cadherin polarized Golgi trafficking to promote invasive breast cancer programs. EMBO Rep 17(7):1061–1080. https://doi.org/10.15252/embr.201642032

Kermorgant S, Parker PJ (2008) Receptor trafficking controls weak signal delivery: a strategy used by c-Met for STAT3 nuclear accumulation. J Cell Biol 182(5):855–863. https://doi.org/10.1083/jcb.200806076

Kiyokawa E, Hara S, Nakamura T, Matsuda M (2006) Fluorescence (Forster) resonance energy transfer imaging of oncogene activity in living cells. Cancer Sci 97(1):8–15. https://doi.org/10.1111/j.1349-7006.2006.00141.x

Kunita R, Otomo A, Mizumura H, Suzuki-Utsunomiya K, Hadano S, Ikeda JE (2007) The Rab5 activator ALS2/alsin acts as a novel Rac1 effector through Rac1-activated endocytosis. J Biol Chem 282(22):16599–16611. https://doi.org/10.1074/jbc.M610682200

Lamaze C, Chuang TH, Terlecky LJ, Bokoch GM, Schmid SL (1996) Regulation of receptor-mediated endocytosis by Rho and Rac. Nature 382(6587):177–179. https://doi.org/10.1038/382177a0

Lamaze C, Dujeancourt A, Baba T, Lo CG, Benmerah A, Dautry-Varsat A (2001) Interleukin 2 receptors and detergent-resistant membrane domains define a clathrin-independent endocytic pathway. Mol Cell 7(3):661–671

Lambert JM, Lambert QT, Reuther GW, Malliri A, Siderovski DP, Sondek J, Collard JG, Der CJ (2002) Tiam1 mediates Ras activation of Rac by a PI(3)K-independent mechanism. Nat Cell Biol 4(8):621–625. https://doi.org/10.1038/ncb833

Lanzetti L, Di Fiore PP (2008) Endocytosis and cancer: an 'insider' network with dangerous liaisons. Traffic 9(12):2011–2021. https://doi.org/10.1111/j.1600-0854.2008.00816.x

Laude AJ, Prior IA (2008) Palmitoylation and localisation of RAS isoforms are modulated by the hypervariable linker domain. J Cell Sci 121(Pt 4):421–427. https://doi.org/10.1242/jcs.020107

Le Roy C, Wrana JL (2005) Clathrin- and non-clathrin-mediated endocytic regulation of cell signalling. Nat Rev Mol Cell Biol 6(2):112–126. https://doi.org/10.1038/nrm1571

Leventis R, Silvius JR (1998) Lipid-binding characteristics of the polybasic carboxy-terminal sequence of K-ras4B. Biochemistry 37(20):7640–7648. https://doi.org/10.1021/bi973077h

Li S, Wang Q, Wang Y, Chen X, Wang Z (2009) PLC-gamma1 and Rac1 coregulate EGF-induced cytoskeleton remodeling and cell migration. Mol Endocrinol 23(6):901–913. https://doi.org/10.1210/me.2008-0368

Llado A, Tebar F, Calvo M, Moreto J, Sorkin A, Enrich C (2004) Protein kinaseCdelta-calmodulin crosstalk regulates epidermal growth factor receptor exit from early endosomes. Mol Biol Cell 15(11):4877–4891. https://doi.org/10.1091/mbc.E04-02-0127

Llado A, Timpson P, Vila de Muga S, Moreto J, Pol A, Grewal T, Daly RJ, Enrich C, Tebar F (2008) Protein kinase Cdelta and calmodulin regulate epidermal growth factor receptor recycling from early endosomes through Arp2/3 complex and cortactin. Mol Biol Cell 19(1):17–29. https://doi.org/10.1091/mbc.E07-05-0411

Lobert VH, Stenmark H (2011) Cell polarity and migration: emerging role for the endosomal sorting machinery. Physiology (Bethesda) 26(3):171–180. https://doi.org/10.1152/physiol.00054.2010

Lopez-Alcala C, Alvarez-Moya B, Villalonga P, Calvo M, Bachs O, Agell N (2008) Identification of essential interacting elements in K-Ras/calmodulin binding and its role in K-Ras localization. J Biol Chem 283(16):10621–10631. https://doi.org/10.1074/jbc.M706238200

Lu A, Tebar F, Alvarez-Moya B, Lopez-Alcala C, Calvo M, Enrich C, Agell N, Nakamura T, Matsuda M, Bachs O (2009) A clathrin-dependent pathway leads to KRas signaling on late endosomes en route to lysosomes. J Cell Biol 184(6):863–879. https://doi.org/10.1083/jcb.200807186

Luo Y, Cheng Z, Dixon CJ, Hall JF, Taylor E, Boarder MR (2011) Endosomal signalling of epidermal growth factor receptors contributes to EGF-stimulated cell cycle progression in primary hepatocytes. Eur J Pharmacol 654(2):173–180. https://doi.org/10.1016/j.ejphar.2010.11.038

Magee T, Marshall C (1999) New insights into the interaction of Ras with the plasma membrane. Cell 98(1):9–12. https://doi.org/10.1016/S0092-8674(00)80601-7

Malliri A, van der Kammen RA, Clark K, van der Valk M, Michiels F, Collard JG (2002) Mice deficient in the Rac activator Tiam1 are resistant to Ras-induced skin tumours. Nature 417(6891):867–871. https://doi.org/10.1038/nature00848

Malumbres M, Barbacid M (2003) RAS oncogenes: the first 30 years. Nat Rev Cancer 3(6):459–465. https://doi.org/10.1038/nrc1097

Marcos-Ramiro B, Garcia-Weber D, Barroso S, Feito J, Ortega MC, Cernuda-Morollon E, Reglero-Real N, Fernandez-Martin L, Duran MC, Alonso MA, Correas I, Cox S, Ridley AJ, Millan

J (2016) RhoB controls endothelial barrier recovery by inhibiting Rac1 trafficking to the cell border. J Cell Biol 213(3):385–402. https://doi.org/10.1083/jcb.201504038
Marei H, Malliri A (2016) Rac1 in human diseases: the therapeutic potential of targeting Rac1 signaling regulatory mechanisms. Small GTPases 1–25. https://doi.org/10.1080/21541248.2016.1211398
Margiotta A, Progida C, Bakke O, Bucci C (2017) Rab7a regulates cell migration through Rac1 and vimentin. Biochim Biophys Acta 2(1864):367–381. https://doi.org/10.1016/j.bbamcr.2016.11.020
Marsh M, Bolzau E, Helenius A (1983) Penetration of Semliki Forest virus from acidic prelysosomal vacuoles. Cell 32(3):931–940
Marshall CJ (1996) Ras effectors. Curr Opin Cell Biol 8(2):197–204
Mayor S, Pagano RE (2007) Pathways of clathrin-independent endocytosis. Nat Rev Mol Cell Biol 8(8):603–612. https://doi.org/10.1038/nrm2216
Mellman I, Yarden Y (2013) Endocytosis and cancer. Cold Spring Harb Perspect Biol 5(12):a016949. https://doi.org/10.1101/cshperspect.a016949
Menard L, Parker PJ, Kermorgant S (2014) Receptor tyrosine kinase c-Met controls the cytoskeleton from different endosomes via different pathways. Nat Commun 5:3907. https://doi.org/10.1038/ncomms4907
Miaczynska M, Bar-Sagi D (2010) Signaling endosomes: seeing is believing. Curr Opin Cell Biol 22(4):535–540. https://doi.org/10.1016/j.ceb.2010.05.007
Miaczynska M, Christoforidis S, Giner A, Shevchenko A, Uttenweiler-Joseph S, Habermann B, Wilm M, Parton RG, Zerial M (2004) APPL proteins link Rab5 to nuclear signal transduction via an endosomal compartment. Cell 116(3):445–456
Michaelson D, Silletti J, Murphy G, D'Eustachio P, Rush M, Philips MR (2001) Differential localization of Rho GTPases in live cells: regulation by hypervariable regions and RhoGDI binding. J Cell Biol 152(1):111–126
Misaki R, Morimatsu M, Uemura T, Waguri S, Miyoshi E, Taniguchi N, Matsuda M, Taguchi T (2010) Palmitoylated Ras proteins traffic through recycling endosomes to the plasma membrane during exocytosis. J Cell Biol 191(1):23–29. https://doi.org/10.1083/jcb.200911143
Mochizuki N, Yamashita S, Kurokawa K, Ohba Y, Nagai T, Miyawaki A, Matsuda M (2001) Spatio-temporal images of growth-factor-induced activation of Ras and Rap1. Nature 411(6841):1065–1068. https://doi.org/10.1038/35082594
Mor A, Philips MR (2006) Compartmentalized Ras/MAPK signaling. Annu Rev Immunol 24:771–800. https://doi.org/10.1146/annurev.immunol.24.021605.090723
Moreto J, Llado A, Vidal-Quadras M, Calvo M, Pol A, Enrich C, Tebar F (2008) Calmodulin modulates H-Ras mediated Raf-1 activation. Cell Signal 20(6):1092–1103. https://doi.org/10.1016/j.cellsig.2008.01.022
Moreto J, Vidal-Quadras M, Pol A, Santos E, Grewal T, Enrich C, Tebar F (2009) Differential involvement of H- and K-Ras in Raf-1 activation determines the role of calmodulin in MAPK signaling. Cell Signal 21(12):1827–1836. https://doi.org/10.1016/j.cellsig.2009.07.018
Murga C, Zohar M, Teramoto H, Gutkind JS (2002) Rac1 and RhoG promote cell survival by the activation of PI3K and Akt, independently of their ability to stimulate JNK and NF-kappaB. Oncogene 21(2):207–216. https://doi.org/10.1038/sj.onc.1205036
Murphy JE, Padilla BE, Hasdemir B, Cottrell GS, Bunnett NW (2009) Endosomes: a legitimate platform for the signaling train. Proc Natl Acad Sci U S A 106(42):17615–17622. https://doi.org/10.1073/pnas.0906541106
Nada S, Hondo A, Kasai A, Koike M, Saito K, Uchiyama Y, Okada M (2009) The novel lipid raft adaptor p18 controls endosome dynamics by anchoring the MEK-ERK pathway to late endosomes. EMBO J 28(5):477–489. https://doi.org/10.1038/emboj.2008.308
Nancy V, Callebaut I, El Marjou A, de Gunzburg J (2002) The delta subunit of retinal rod cGMP phosphodiesterase regulates the membrane association of Ras and Rap GTPases. J Biol Chem 277(17):15076–15084. https://doi.org/10.1074/jbc.M109983200

Navarro-Lerida I, Sanchez-Perales S, Calvo M, Rentero C, Zheng Y, Enrich C, Del Pozo MA (2012) A palmitoylation switch mechanism regulates Rac1 function and membrane organization. EMBO J 31(3):534–551. https://doi.org/10.1038/emboj.2011.446

Newlaczyl AU, Hood FE, Coulson JM, Prior IA (2014) Decoding RAS isoform and codon-specific signalling. Biochem Soc Trans 42(4):742–746. https://doi.org/10.1042/BST20140057

Niv H, Gutman O, Henis YI, Kloog Y (1999) Membrane interactions of a constitutively active GFP-Ki-Ras 4B and their role in signaling. Evidence from lateral mobility studies. J Biol Chem 274(3):1606–1613

Niv H, Gutman O, Kloog Y, Henis YI (2002) Activated K-Ras and H-Ras display different interactions with saturable nonraft sites at the surface of live cells. J Cell Biol 157(5):865–872. https://doi.org/10.1083/jcb.200202009

Ohashi E, Tanabe K, Henmi Y, Mesaki K, Kobayashi Y, Takei K (2011) Receptor sorting within endosomal trafficking pathway is facilitated by dynamic actin filaments. PLoS ONE 6(5):e19942. https://doi.org/10.1371/journal.pone.0019942

Olofsson B (1999) Rho guanine dissociation inhibitors: pivotal molecules in cellular signalling. Cell Signal 11(8):545–554

Omerovic J, Hammond DE, Clague MJ, Prior IA (2008) Ras isoform abundance and signalling in human cancer cell lines. Oncogene 27(19):2754–2762. https://doi.org/10.1038/sj.onc.1210925

Onken B, Wiener H, Philips MR, Chang EC (2006) Compartmentalized signaling of Ras in fission yeast. Proc Natl Acad Sci U S A 103(24):9045–9050. https://doi.org/10.1073/pnas.0603318103

Palamidessi A, Frittoli E, Garre M, Faretta M, Mione M, Testa I, Diaspro A, Lanzetti L, Scita G, Di Fiore PP (2008) Endocytic trafficking of Rac is required for the spatial restriction of signaling in cell migration. Cell 134(1):135–147. https://doi.org/10.1016/j.cell.2008.05.034

Parri M, Chiarugi P (2010) Rac and Rho GTPases in cancer cell motility control. Cell Commun Signal 8:23. https://doi.org/10.1186/1478-811X-8-23

Peranen J, Auvinen P, Virta H, Wepf R, Simons K (1996) Rab8 promotes polarized membrane transport through reorganization of actin and microtubules in fibroblasts. J Cell Biol 135(1):153–167

Pfeffer SR (2009) Multiple routes of protein transport from endosomes to the trans Golgi network. FEBS Lett 583(23):3811–3816. https://doi.org/10.1016/j.febslet.2009.10.075

Philips MR (2012) Ras hitchhikes on PDE6delta. Nat Cell Biol 14(2):128–129. https://doi.org/10.1038/ncb2429

Pinilla-Macua I, Watkins SC, Sorkin A (2016) Endocytosis separates EGF receptors from endogenous fluorescently labeled HRas and diminishes receptor signaling to MAP kinases in endosomes. Proc Natl Acad Sci U S A 113(8):2122–2127. https://doi.org/10.1073/pnas.1520301113

Platta HW, Stenmark H (2011) Endocytosis and signaling. Curr Opin Cell Biol 23(4):393–403. https://doi.org/10.1016/j.ceb.2011.03.008

Pol A, Calvo M, Enrich C (1998) Isolated endosomes from quiescent rat liver contain the signal transduction machinery. Differential distribution of activated Raf-1 and Mek in the endocytic compartment. FEBS Lett 441(1):34–38

Porat-Shliom N, Kloog Y, Donaldson JG (2008) A unique platform for H-Ras signaling involving clathrin-independent endocytosis. Mol Biol Cell 19(3):765–775. https://doi.org/10.1091/mbc.E07-08-0841

Porther N, Barbieri MA (2015) The role of endocytic Rab GTPases in regulation of growth factor signaling and the migration and invasion of tumor cells. Small GTPases 6(3):135–144. https://doi.org/10.1080/21541248.2015.1050152

Posner BI, Patel B, Verma AK, Bergeron JJ (1980) Uptake of insulin by plasmalemma and Golgi subcellular fractions of rat liver. J Biol Chem 255(2):735–741

Poteryaev D, Datta S, Ackema K, Zerial M, Spang A (2010) Identification of the switch in early-to-late endosome transition. Cell 141(3):497–508. https://doi.org/10.1016/j.cell.2010.03.011

Price LS, Langeslag M, ten Klooster JP, Hordijk PL, Jalink K, Collard JG (2003) Calcium signaling regulates translocation and activation of Rac. J Biol Chem 278(41):39413–39421. https://doi.org/10.1074/jbc.M302083200

Prior IA, Hancock JF (2012) Ras trafficking, localization and compartmentalized signalling. Semin Cell Dev Biol 23(2):145–153. https://doi.org/10.1016/j.semcdb.2011.09.002

Prior IA, Muncke C, Parton RG, Hancock JF (2003) Direct visualization of Ras proteins in spatially distinct cell surface microdomains. J Cell Biol 160(2):165–170. https://doi.org/10.1083/jcb.200209091

Puthenveedu MA, Lauffer B, Temkin P, Vistein R, Carlton P, Thorn K, Taunton J, Weiner OD, Parton RG, von Zastrow M (2010) Sequence-dependent sorting of recycling proteins by actin-stabilized endosomal microdomains. Cell 143(5):761–773. https://doi.org/10.1016/j.cell.2010.10.003

Qiu RG, Chen J, McCormick F, Symons M (1995) A role for Rho in Ras transformation. Proc Natl Acad Sci U S A 92(25):11781–11785

Rajalingam K, Schreck R, Rapp UR, Albert S (2007) Ras oncogenes and their downstream targets. Biochim Biophys Acta 1773(8):1177–1195. https://doi.org/10.1016/j.bbamcr.2007.01.012

Reid TS, Terry KL, Casey PJ, Beese LS (2004) Crystallographic analysis of CaaX prenyltransferases complexed with substrates defines rules of protein substrate selectivity. J Mol Biol 343(2):417–433. https://doi.org/10.1016/j.jmb.2004.08.056

Revach OY, Winograd-Katz SE, Samuels Y, Geiger B (2016) The involvement of mutant Rac1 in the formation of invadopodia in cultured melanoma cells. Exp Cell Res 343(1):82–88. https://doi.org/10.1016/j.yexcr.2016.02.003

Reverter M, Rentero C, Garcia-Melero A, Hoque M, Vila de Muga S, Alvarez-Guaita A, Conway JR, Wood P, Cairns R, Lykopoulou L, Grinberg D, Vilageliu L, Bosch M, Heeren J, Blasi J, Timpson P, Pol A, Tebar F, Murray RZ, Grewal T, Enrich C (2014) Cholesterol regulates Syntaxin 6 trafficking at trans-Golgi network endosomal boundaries. Cell Rep 7(3):883–897. https://doi.org/10.1016/j.celrep.2014.03.043

Ridley AJ (2001) Rho GTPases and cell migration. J Cell Sci 114(Pt 15):2713–2722

Ridley AJ (2006) Rho GTPases and actin dynamics in membrane protrusions and vesicle trafficking. Trends Cell Biol 16(10):522–529. https://doi.org/10.1016/j.tcb.2006.08.006

Rink J, Ghigo E, Kalaidzidis Y, Zerial M (2005) Rab conversion as a mechanism of progression from early to late endosomes. Cell 122(5):735–749. https://doi.org/10.1016/j.cell.2005.06.043

Rocks O, Peyker A, Kahms M, Verveer PJ, Koerner C, Lumbierres M, Kuhlmann J, Waldmann H, Wittinghofer A, Bastiaens PI (2005) An acylation cycle regulates localization and activity of palmitoylated Ras isoforms. Science 307(5716):1746–1752. https://doi.org/10.1126/science.1105654

Rocks O, Gerauer M, Vartak N, Koch S, Huang ZP, Pechlivanis M, Kuhlmann J, Brunsveld L, Chandra A, Ellinger B, Waldmann H, Bastiaens PI (2010) The palmitoylation machinery is a spatially organizing system for peripheral membrane proteins. Cell 141(3):458–471. https://doi.org/10.1016/j.cell.2010.04.007

Rojas R, van Vlijmen T, Mardones GA, Prabhu Y, Rojas AL, Mohammed S, Heck AJ, Raposo G, van der Sluijs P, Bonifacino JS (2008) Regulation of retromer recruitment to endosomes by sequential action of Rab5 and Rab7. J Cell Biol 183(3):513–526. https://doi.org/10.1083/jcb.200804048

Rojas AM, Fuentes G, Rausell A, Valencia A (2012) The Ras protein superfamily: evolutionary tree and role of conserved amino acids. J Cell Biol 196(2):189–201. https://doi.org/10.1083/jcb.201103008

Roland JT, Kenworthy AK, Peranen J, Caplan S, Goldenring JR (2007) Myosin Vb interacts with Rab8a on a tubular network containing EHD1 and EHD3. Mol Biol Cell 18(8):2828–2837. https://doi.org/10.1091/mbc.E07-02-0169

Roy S, Wyse B, Hancock JF (2002) H-Ras signaling and K-Ras signaling are differentially dependent on endocytosis. Mol Cell Biol 22(14):5128–5140

Sahai E, Marshall CJ (2002) RHO-GTPases and cancer. Nat Rev Cancer 2(2):133–142. https://doi.org/10.1038/nrc725

Samuel MS, Lourenco FC, Olson MF (2011) K-Ras mediated murine epidermal tumorigenesis is dependent upon and associated with elevated Rac1 activity. PLoS ONE 6(2):e17143. https://doi.org/10.1371/journal.pone.0017143

Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM (2010) Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell 141(2):290–303. https://doi.org/10.1016/j.cell.2010.02.024

Sander EE, van Delft S, ten Klooster JP, Reid T, van der Kammen RA, Michiels F, Collard JG (1998) Matrix-dependent Tiam1/Rac signaling in epithelial cells promotes either cell-cell adhesion or cell migration and is regulated by phosphatidylinositol 3-kinase. J Cell Biol 143(5):1385–1398

Sandri C, Caccavari F, Valdembri D, Camillo C, Veltel S, Santambrogio M, Lanzetti L, Bussolino F, Ivaska J, Serini G (2012) The R-Ras/RIN2/Rab5 complex controls endothelial cell adhesion and morphogenesis via active integrin endocytosis and Rac signaling. Cell Res 22(10):1479–1501. https://doi.org/10.1038/cr.2012.110

Sasaki AT, Carracedo A, Locasale JW, Anastasiou D, Takeuchi K, Kahoud ER, Haviv S, Asara JM, Pandolfi PP, Cantley LC (2011) Ubiquitination of K-Ras enhances activation and facilitates binding to select downstream effectors. Sci Signal 4(163):ra13. https://doi.org/10.1126/scisignal.2001518

Sauvonnet N, Dujeancourt A, Dautry-Varsat A (2005) Cortactin and dynamin are required for the clathrin-independent endocytosis of gammac cytokine receptor. J Cell Biol 168(1):155–163. https://doi.org/10.1083/jcb.200406174

Schafer DA, Weed SA, Binns D, Karginov AV, Parsons JT, Cooper JA (2002) Dynamin2 and cortactin regulate actin assembly and filament organization. Curr Biol 12(21):1852–1857

Schenck A, Goto-Silva L, Collinet C, Rhinn M, Giner A, Habermann B, Brand M, Zerial M (2008) The endosomal protein Appl1 mediates Akt substrate specificity and cell survival in vertebrate development. Cell 133(3):486–497. https://doi.org/10.1016/j.cell.2008.02.044

Schiefermeier N, Scheffler JM, de Araujo ME, Stasyk T, Yordanov T, Ebner HL, Offterdinger M, Munck S, Hess MW, Wickstrom SA, Lange A, Wunderlich W, Fassler R, Teis D, Huber LA (2014) The late endosomal p14-MP1 (LAMTOR2/3) complex regulates focal adhesion dynamics during cell migration. J Cell Biol 205(4):525–540. https://doi.org/10.1083/jcb.201310043

Schmick M, Kraemer A, Bastiaens PI (2015) Ras moves to stay in place. Trends Cell Biol 25(4):190–197. https://doi.org/10.1016/j.tcb.2015.02.004

Schnelzer A, Prechtel D, Knaus U, Dehne K, Gerhard M, Graeff H, Harbeck N, Schmitt M, Lengyel E (2000) Rac1 in human breast cancer: overexpression, mutation analysis, and characterization of a new isoform, Rac1b. Oncogene 19(26):3013–3020. https://doi.org/10.1038/sj.onc.1203621

Scita G, Di Fiore PP (2010) The endocytic matrix. Nature 463(7280):464–473. https://doi.org/10.1038/nature08910

Scott CC, Vacca F, Gruenberg J (2014) Endosome maturation, transport and functions. Semin Cell Dev Biol 31:2–10. https://doi.org/10.1016/j.semcdb.2014.03.034

Shafaq-Zadah M, Gomes-Santos CS, Bardin S, Maiuri P, Maurin M, Iranzo J, Gautreau A, Lamaze C, Caswell P, Goud B, Johannes L (2016) Persistent cell migration and adhesion rely on retrograde transport of beta(1) integrin. Nat Cell Biol 18(1):54–64. https://doi.org/10.1038/ncb3287

Shenoy SK, Lefkowitz RJ (2011) Beta-Arrestin-mediated receptor trafficking and signal transduction. Trends Pharmacol Sci 32(9):521–533. https://doi.org/10.1016/j.tips.2011.05.002

Shibasaki Y, Ishihara H, Kizuki N, Asano T, Oka Y, Yazaki Y (1997) Massive actin polymerization induced by phosphatidylinositol-4-phosphate 5-kinase in vivo. J Biol Chem 272(12):7578–7581

Sigismund S, Argenzio E, Tosoni D, Cavallaro E, Polo S, Di Fiore PP (2008) Clathrin-mediated internalization is essential for sustained EGFR signaling but dispensable for degradation. Dev Cell 15(2):209–219. https://doi.org/10.1016/j.devcel.2008.06.012

Sigismund S, Confalonieri S, Ciliberto A, Polo S, Scita G, Di Fiore PP (2012) Endocytosis and signaling: cell logistics shape the eukaryotic cell plan. Physiol Rev 92(1):273–366. https://doi.org/10.1152/physrev.00005.2011

Silvius JR, Bhagatji P, Leventis R, Terrone D (2006) K-ras4B and prenylated proteins lacking "second signals" associate dynamically with cellular membranes. Mol Biol Cell 17(1):192–202. https://doi.org/10.1091/mbc.E05-05-0408

Soriano-Castell D, Chavero A, Rentero C, Bosch M, Vidal-Quadras M, Pol A, Enrich C, Tebar F (2017) ROCK1 is a novel Rac1 effector to regulate tubular endocytic membrane formation during

clathrinindependent endocytosis. Sci Rep 7(1):6866. https://doi.org/10.1038/s41598-017-07130-x
Sorkin A, von Zastrow M (2002) Signal transduction and endocytosis: close encounters of many kinds. Nat Rev Mol Cell Biol 3(8):600–614. https://doi.org/10.1038/nrm883
Sorkin A, von Zastrow M (2009) Endocytosis and signalling: intertwining molecular networks. Nat Rev Mol Cell Biol 10(9):609–622. https://doi.org/10.1038/nrm2748
Stasyk T, Huber LA (2016) Spatio-temporal parameters of endosomal signaling in cancer: implications for new treatment options. J Cell Biochem 117(4):836–843. https://doi.org/10.1002/jcb.25418
Sturge J, Wienke D, Isacke CM (2006) Endosomes generate localized Rho-ROCK-MLC2-based contractile signals via Endo180 to promote adhesion disassembly. J Cell Biol 175(2):337–347. https://doi.org/10.1083/jcb.200602125
Sugihara K, Nakatsuji N, Nakamura K, Nakao K, Hashimoto R, Otani H, Sakagami H, Kondo H, Nozawa S, Aiba A, Katsuki M (1998) Rac1 is required for the formation of three germ layers during gastrulation. Oncogene 17(26):3427–3433. https://doi.org/10.1038/sj.onc.1202595
Sun Y, Buki KG, Ettala O, Vaaraniemi JP, Vaananen HK (2005) Possible role of direct Rac1-Rab7 interaction in ruffled border formation of osteoclasts. J Biol Chem 280(37):32356–32361. https://doi.org/10.1074/jbc.M414213200
Swarthout JT, Lobo S, Farh L, Croke MR, Greentree WK, Deschenes RJ, Linder ME (2005) DHHC9 and GCP16 constitute a human protein fatty acyltransferase with specificity for H- and N-Ras. J Biol Chem 280(35):31141–31148. https://doi.org/10.1074/jbc.M504113200
Tall GG, Barbieri MA, Stahl PD, Horazdovsky BF (2001) Ras-activated endocytosis is mediated by the Rab5 guanine nucleotide exchange activity of RIN1. Dev Cell 1(1):73–82
Taub N, Teis D, Ebner HL, Hess MW, Huber LA (2007) Late endosomal traffic of the epidermal growth factor receptor ensures spatial and temporal fidelity of mitogen-activated protein kinase signaling. Mol Biol Cell 18(12):4698–4710. https://doi.org/10.1091/mbc.E07-02-0098
Tebar F, Villalonga P, Sorkina T, Agell N, Sorkin A, Enrich C (2002) Calmodulin regulates intracellular trafficking of epidermal growth factor receptor and the MAPK signaling pathway. Mol Biol Cell 13(6):2057–2068. https://doi.org/10.1091/mbc.01-12-0571
Teis D, Huber LA (2003) The odd couple: signal transduction and endocytosis. Cell Mol Life Sci 60(10):2020–2033. https://doi.org/10.1007/s00018-003-3010-2
Teis D, Wunderlich W, Huber LA (2002) Localization of the MP1-MAPK scaffold complex to endosomes is mediated by p14 and required for signal transduction. Dev Cell 3(6):803–814
Teis D, Taub N, Kurzbauer R, Hilber D, de Araujo ME, Erlacher M, Offterdinger M, Villunger A, Geley S, Bohn G, Klein C, Hess MW, Huber LA (2006) p14-MP1-MEK1 signaling regulates endosomal traffic and cellular proliferation during tissue homeostasis. J Cell Biol 175(6):861–868. https://doi.org/10.1083/jcb.200607025
ten Klooster JP, Hordijk PL (2007) Targeting and localized signalling by small GTPases. Biol Cell 99(1):1–12. https://doi.org/10.1042/BC20060071
ten Klooster JP, Jaffer ZM, Chernoff J, Hordijk PL (2006) Targeting and activation of Rac1 are mediated by the exchange factor beta-Pix. J Cell Biol 172(5):759–769. https://doi.org/10.1083/jcb.200509096
Tolias K, Carpenter CL (2000) In vitro interaction of phosphoinositide-4-phosphate 5-kinases with Rac. Methods Enzymol 325:190–200
Tolias KF, Hartwig JH, Ishihara H, Shibasaki Y, Cantley LC, Carpenter CL (2000) Type Ialpha phosphatidylinositol-4-phosphate 5-kinase mediates Rac-dependent actin assembly. Curr Biol 10(3):153–156
Tomas A, Futter CE, Eden ER (2014) EGF receptor trafficking: consequences for signaling and cancer. Trends Cell Biol 24(1):26–34. https://doi.org/10.1016/j.tcb.2013.11.002
Tsukazaki T, Chiang TA, Davison AF, Attisano L, Wrana JL (1998) SARA, a FYVE domain protein that recruits Smad2 to the TGFbeta receptor. Cell 95(6):779–791

Tsutsumi K, Fujioka Y, Tsuda M, Kawaguchi H, Ohba Y (2009) Visualization of Ras-PI3K interaction in the endosome using BiFC. Cell Signal 21(11):1672–1679. https://doi.org/10.1016/j.cellsig.2009.07.004

Valdez G, Philippidou P, Rosenbaum J, Akmentin W, Shao Y, Halegoua S (2007) Trk-signaling endosomes are generated by Rac-dependent macroendocytosis. Proc Natl Acad Sci U S A 104(30):12270–12275. https://doi.org/10.1073/pnas.0702819104

van den Bout I, Divecha N (2009) PIP5K-driven PtdIns(4,5)P_2 synthesis: regulation and cellular functions. J Cell Sci 122(Pt 21):3837–3850. https://doi.org/10.1242/jcs.056127

Vartak N, Bastiaens P (2010) Spatial cycles in G-protein crowd control. EMBO J 29(16):2689–2699. https://doi.org/10.1038/emboj.2010.184

Vidal C, Geny B, Melle J, Jandrot-Perrus M, Fontenay-Roupie M (2002) Cdc42/Rac1-dependent activation of the p21-activated kinase (PAK) regulates human platelet lamellipodia spreading: implication of the cortical-actin binding protein cortactin. Blood 100(13):4462–4469. https://doi.org/10.1182/blood.V100.13.4462

Vidal-Quadras M, Gelabert-Baldrich M, Soriano-Castell D, Llado A, Rentero C, Calvo M, Pol A, Enrich C, Tebar F (2011) Rac1 and calmodulin interactions modulate dynamics of ARF6-dependent endocytosis. Traffic 12(12):1879–1896. https://doi.org/10.1111/j.1600-0854.2011.01274.x

Vieira AV, Lamaze C, Schmid SL (1996) Control of EGF receptor signaling by clathrin-mediated endocytosis. Science 274(5295):2086–2089

Vigil D, Cherfils J, Rossman KL, Der CJ (2010) Ras superfamily GEFs and GAPs: validated and tractable targets for cancer therapy? Nat Rev Cancer 10(12):842–857. https://doi.org/10.1038/nrc2960

Vilardaga JP, Jean-Alphonse FG, Gardella TJ (2014) Endosomal generation of cAMP in GPCR signaling. Nat Chem Biol 10(9):700–706. https://doi.org/10.1038/nchembio.1611

Villalonga P, Lopez-Alcala C, Bosch M, Chiloeches A, Rocamora N, Gil J, Marais R, Marshall CJ, Bachs O, Agell N (2001) Calmodulin binds to K-Ras, but not to H- or N-Ras, and modulates its downstream signaling. Mol Cell Biol 21(21):7345–7354. https://doi.org/10.1128/MCB.21.21.7345-7354.2001

Villasenor R, Kalaidzidis Y, Zerial M (2016) Signal processing by the endosomal system. Curr Opin Cell Biol 39:53–60. https://doi.org/10.1016/j.ceb.2016.02.002

von Zastrow M, Sorkin A (2007) Signaling on the endocytic pathway. Curr Opin Cell Biol 19(4):436–445. https://doi.org/10.1016/j.ceb.2007.04.021

Vonderheit A, Helenius A (2005) Rab7 associates with early endosomes to mediate sorting and transport of Semliki forest virus to late endosomes. PLoS Biol 3(7):e233. https://doi.org/10.1371/journal.pbio.0030233

Walsh AB, Bar-Sagi D (2001) Differential activation of the Rac pathway by Ha-Ras and K-Ras. J Biol Chem 276(19):15609–15615. https://doi.org/10.1074/jbc.M010573200

Wang Y, Pennock S, Chen X, Wang Z (2002) Endosomal signaling of epidermal growth factor receptor stimulates signal transduction pathways leading to cell survival. Mol Cell Biol 22(20):7279–7290

Watson FL, Heerssen HM, Bhattacharyya A, Klesse L, Lin MZ, Segal RA (2001) Neurotrophins use the Erk5 pathway to mediate a retrograde survival response. Nat Neurosci 4(10):981–988. https://doi.org/10.1038/nn720

Webb BA, Zhou S, Eves R, Shen L, Jia L, Mak AS (2006) Phosphorylation of cortactin by p21-activated kinase. Arch Biochem Biophys 456(2):183–193. https://doi.org/10.1016/j.abb.2006.06.011

Weernink PA, Meletiadis K, Hommeltenberg S, Hinz M, Ishihara H, Schmidt M, Jakobs KH (2004) Activation of type I phosphatidylinositol 4-phosphate 5-kinase isoforms by the Rho GTPases, RhoA, Rac1, and Cdc42. J Biol Chem 279(9):7840–7849. https://doi.org/10.1074/jbc.M312737200

White IJ, Bailey LM, Aghakhani MR, Moss SE, Futter CE (2006) EGF stimulates annexin 1-dependent inward vesiculation in a multivesicular endosome subpopulation. EMBO J 25(1):1–12. https://doi.org/10.1038/sj.emboj.7600759

Wiesner C, El Azzouzi K, Linder S (2013) A specific subset of RabGTPases controls cell surface exposure of MT1-MMP, extracellular matrix degradation and three-dimensional invasion of macrophages. J Cell Sci 126(Pt 13):2820–2833. https://doi.org/10.1242/jcs.122358

Williams KC, Coppolino MG (2011) Phosphorylation of membrane type 1-matrix metalloproteinase (MT1-MMP) and its vesicle-associated membrane protein 7 (VAMP7)-dependent trafficking facilitate cell invasion and migration. J Biol Chem 286(50):43405–43416. https://doi.org/10.1074/jbc.M111.297069

Wittinghofer A, Herrmann C (1995) Ras-effector interactions, the problem of specificity. FEBS Lett 369(1):52–56

Wittinghofer A, Vetter IR (2011) Structure-function relationships of the G domain, a canonical switch motif. Annu Rev Biochem 80:943–971. https://doi.org/10.1146/annurev-biochem-062708-134043

Wu C, Lai CF, Mobley WC (2001) Nerve growth factor activates persistent Rap1 signaling in endosomes. J Neurosci 21(15):5406–5416

Xu L, Lubkov V, Taylor LJ, Bar-Sagi D (2010) Feedback regulation of Ras signaling by Rabex-5-mediated ubiquitination. Curr Biol 20(15):1372–1377. https://doi.org/10.1016/j.cub.2010.06.051

Yan H, Jahanshahi M, Horvath EA, Liu HY, Pfleger CM (2010) Rabex-5 ubiquitin ligase activity restricts Ras signaling to establish pathway homeostasis in Drosophila. Curr Biol 20(15):1378–1382. https://doi.org/10.1016/j.cub.2010.06.058

Yang N, Higuchi O, Ohashi K, Nagata K, Wada A, Kangawa K, Nishida E, Mizuno K (1998) Cofilin phosphorylation by LIM-kinase 1 and its role in Rac-mediated actin reorganization. Nature 393(6687):809–812. https://doi.org/10.1038/31735

Yang Y, Du J, Hu Z, Liu J, Tian Y, Zhu Y, Wang L, Gu L (2011) Activation of Rac1-PI3K/Akt is required for epidermal growth factor-induced PAK1 activation and cell migration in MDA-MB-231 breast cancer cells. J Biomed Res 25(4):237–245. https://doi.org/10.1016/S1674-8301(11)60032-8

Yeung T, Terebiznik M, Yu L, Silvius J, Abidi WM, Philips M, Levine T, Kapus A, Grinstein S (2006) Receptor activation alters inner surface potential during phagocytosis. Science 313(5785):347–351. https://doi.org/10.1126/science.1129551

Yeung T, Gilbert GE, Shi J, Silvius J, Kapus A, Grinstein S (2008) Membrane phosphatidylserine regulates surface charge and protein localization. Science 319(5860):210–213. https://doi.org/10.1126/science.1152066

Yokoe H, Meyer T (1996) Spatial dynamics of GFP-tagged proteins investigated by local fluorescence enhancement. Nat Biotechnol 14(10):1252–1256. https://doi.org/10.1038/nbt1096-1252

Zerial M, McBride H (2001) Rab proteins as membrane organizers. Nat Rev Mol Cell Biol 2(2):107–117. https://doi.org/10.1038/35052055

Zheng ZY, Chang EC (2014) A bimolecular fluorescent complementation screen reveals complex roles of endosomes in Ras-mediated signaling. Methods Enzymol 535:25–38. https://doi.org/10.1016/B978-0-12-397925-4.00002-X

Zheng ZY, Cheng CM, Fu XR, Chen LY, Xu L, Terrillon S, Wong ST, Bar-Sagi D, Songyang Z, Chang EC (2012a) CHMP6 and VPS4A mediate the recycling of Ras to the plasma membrane to promote growth factor signaling. Oncogene 31(43):4630–4638. https://doi.org/10.1038/onc.2011.607

Zheng ZY, Xu L, Bar-Sagi D, Chang EC (2012b) Escorting Ras. Small GTPases 3(4):236–239. https://doi.org/10.4161/sgtp.20460

Zhou Y, Hancock JF (2015) Ras nanoclusters: Versatile lipid-based signaling platforms. Biochim Biophys Acta 1853(4):841–849. https://doi.org/10.1016/j.bbamcr.2014.09.008

Zhou P, Porcionatto M, Pilapil M, Chen Y, Choi Y, Tolias KF, Bikoff JB, Hong EJ, Greenberg ME, Segal RA (2007) Polarized signaling endosomes coordinate BDNF-induced chemotaxis of cerebellar precursors. Neuron 55(1):53–68. https://doi.org/10.1016/j.neuron.2007.05.030

Zhu G, Fan Z, Ding M, Zhang H, Mu L, Ding Y, Zhang Y, Jia B, Chen L, Chang Z, Wu W (2015) An EGFR/PI3K/AKT axis promotes accumulation of the Rac1-GEF Tiam1 that is critical in EGFR-driven tumorigenesis. Oncogene 34(49):5971–5982. https://doi.org/10.1038/onc.2015.45

Zoncu R, Perera RM, Balkin DM, Pirruccello M, Toomre D, De Camilli P (2009) A phosphoinositide switch controls the maturation and signaling properties of APPL endosomes. Cell 136(6):1110–1121. https://doi.org/10.1016/j.cell.2009.01.032

Zoncu R, Efeyan A, Sabatini DM (2011) mTOR: from growth signal integration to cancer, diabetes and ageing. Nat Rev Mol Cell Biol 12(1):21–35. https://doi.org/10.1038/nrm3025