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REVIEW

The EGFR odyssey – from activation to destruction in space and time

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ABSTRACT

When cell surface receptors engage their cognate ligands in the extracellular space, they become competent to transmit potent signals to the inside of the cell, thereby instigating growth, differentiation, motility and many other processes. In order to control these signals, activated receptors are endocytosed and thoroughly curated by the endosomal network of intracellular vesicles and proteolytic organelles. In this Review, we follow the epidermal growth factor (EGF) receptor (EGFR) from ligand engagement, through its voyage on endosomes and, ultimately, to its destruction in the lysosome. We focus on the spatial and temporal considerations underlying the molecular decisions that govern this complex journey and discuss how additional cellular organelles – particularly the ER – play active roles in the regulation of receptor lifespan. In summarizing the functions of relevant molecules on the endosomes and the ER, we cover the order of molecular events in receptor activation, trafficking and downregulation, and provide an overview of how signaling is controlled at the interface between these organelles.

KEY WORDS: EGFR, Endosomes, ER, Signaling, Rab7, Rab5

Introduction

Multicellular life necessitates communication between distantly located cells in a manner that is straightforward to initiate, decode and act upon. To serve these universal needs, cell surface receptors have evolved to recognize and respond to environmental cues with exquisite specificity and precision. In mammalian cells, some of the most vital cellular signaling pathways, including proliferation and differentiation, fall under the purview of growth factor receptors. Imbedded in the plasma membrane, these proteins extend ligand-interacting sensory platforms into the extracellular space and receptor tyrosine kinase (RTK) response modules into the cytosol. This arrangement couples environmental inputs received via growth factor binding to signaling cascades transduced inside the cell upon kinase activation. Because stimulatory ligands for these receptors are produced at a distance, their activation is inducible on demand. Crucially, once the receptors become turned 'on', their signals must be terminated in order for cells to regain equilibrium and maintain responsiveness to future inputs. This balance between activation and downregulation is managed largely by the uptake of receptors from the cell surface into the vesicular network of the endocytic pathway, where timing and directionality of transport modulate signal duration and determine receptor fate. Adding further complexity to the matter, receptors such as epidermal growth factor (EGF) receptor (EGFR) signal not only at the cell surface, where ligand engagement occurs, but continue signaling on endosomes for a comparable period (Haugh et al., 1999; Leonard et al., 2008; Foley et al., 2012; Francavilla et al., 2016). EGFR has also been reported to localize to the nucleus, where it is suggested to function as a transcription factor that is associated with cancer disease progression (Kamio et al., 1990; Brand et al., 2013). From ligand encounters to receptor degradation in the lysosome, in this Review, we discuss how EGFR navigates the endosomal system, toggling its signaling switch in cellular space and time.

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What happens at the cell surface (doesn't always stay there)

EGFR – the model RTK

EGFR is the first identified member of the receptor tyrosine kinase (RTK) family (Burgess et al., 2003; Bublil and Yarden, 2007) and, in accordance with its plethora of functions, is expressed on the surface of numerous cell types (Chen et al., 2016). When in its active or 'on' state, EGFR transduces signals to the cell interior that instigate key processes of life, such as growth, differentiation, proliferation and motility (Ceresa and Peterson, 2014; Li et al., 2017). Given these profound effects, the association of EGFR with cancer is self-evident and exemplified by the vast number of studies that link deregulated expression and degradation of EGFR, as well as its activating mutations, with transformation (Shan et al., 2012; Tomas et al., 2014). Because many of the basic principles of EGFR biology are shared by its lesser-studied family members and beyond, EGFR represents the model growth factor RTK.

Activate me

EGFR can be activated by a number of ligands, of which EGF is most extensively studied (Cohen, 1962; Cohen and Carpenter, 1975; Harris et al., 2003; Singh et al., 2016). These ligands are produced as transmembrane precursors whose juxtacrine, paracrine and/or endocrine origins vary depending on the biological cues that instigate activity of EGFR. Typically, EGF production is locally controlled, as opposed to being delivered systemically, such as in the case of hormones, which makes it possible for different organs to conduct their own EGF-mediated programs (Singh and Harris, 2005; Conte and Sigismund, 2016). Once released into the extracellular milieu, EGF and related ligands begin the search for their cognate receptors, thereby setting in motion cellular programs of survival and growth (Massague and Pandiella, 1993; Sahin et al., 2004; Li et al., 2015; Chen et al., 2017). Specificity of EGFR activation is mediated through the establishment of defined contacts between the ligand and the binding groove of the receptor located on its extracellular face (Bajaj et al., 1987; Lax et al., 1988; Ferguson et al., 2003; Jorissen et al., 2003; Zhu et al., 2017). Variations in sidechain features between different ligands, as well as post-translational modifications present on the extracellular EGFR domain, determine the strength of engagement (Azimzadeh Irani et al., 2017). Solid-state nuclear magnetic resonance (NMR)

experiments have demonstrated that, in the absence of ligand, the intracellular region of EGFR exists in a rigid conformation, while the extracellular domain remains highly dynamic. Ligand binding sharply restricts this flexibility, providing a stable platform for ligand-mediated dimerization – a key event in receptor activation and initiation of downstream signaling (Ogiso et al., 2002; Kaplan et al., 2016). Within the receptor dimer, rotation of the transmembrane segment transduces a conformational change to the intracellular kinase domains, resulting in their asymmetric positioning, which in turn promotes cross-phosphorylation of cytoplasmic receptor tails (Honegger et al., 1989; Moriki et al., 2001; Kourouniotis et al., 2016; Purba et al., 2017). Depending on the type and degree of phosphorylation, the latter can now recruit specific signaling complexes and thus have the potential to initiate a wide variety of downstream signaling cascades associated with EGF-dependent responses (Foley et al., 2012; Wagner et al., 2013; Ceresa and Peterson, 2014; Li et al., 2017).

Ligand or not

In the absence of ligand, most EGFR molecules remain in their monomeric form and are therefore inactive. However, because the arrival of any external signals is difficult to anticipate, EGFR has evolved to be intrinsically poised towards the 'on' state, occasionally giving rise to auto-activation (Ferguson et al., 2003; Burgess et al., 2003; Ceresa and Peterson, 2014). Therefore, while maintaining acute responsiveness to ligands, cells must also guard themselves against aberrant or excessive activation of EGFR. These needs are accommodated through continuous surface sampling and the differential intracellular routing of receptors (Fig. 1). Although inactive receptors continuously travel through the endocytic compartment (Fig. 1, step 1+route 1), slow internalization and rapid recycling rates ensure their accumulation on the cell surface. Upon ligand binding (Fig. 1, step 2), this equilibrium shifts rapidly (Herbst et al., 1994; Burke and Wiley, 1999; Wiley, 2003; Ceresa and Peterson, 2014; Tomas et al., 2014), causing activated receptors to spend extended periods of time traveling the endocytic route (Fig. 1, step 3+route 3). In this case, signaling continues until receptors are either recycled back to the cell surface or taken up into proteolytic lysosomes, leading to their demise. Understanding how cells control the duration of legitimate ligand-mediated responses, while keeping unwarranted activation at bay in many ways encompasses the crux of signaling pathways. It appears that cells have taken the 'divide and conquer' approach to solving this problem by segregating the receptor 'on' and 'off' states in cellular space and time. How this is orchestrated to afford proper regulation of EGFR lifespan is discussed in the following sections.

Receptor endocytosis and the peripheral–perinuclear divide

Endosomes – signaling hubs or traps for destruction?

Although key steps in ligand engagement and nucleation of signaling cascades take place at the cell surface, once activated, EGFR molecules actually spend most of their remaining lifetime in the cell interior, traversing the vesicular network of the endosomal system. Under conditions of low ligand availability, activated EGFR is typically subjected to clathrin-mediated endocytosis (CME) (Sigismund et al., 2005; Robinson, 2015). Although inactive EGFR can move into preformed clathrin-coated pits, phosphorylated receptor accelerates CME by attracting the adaptor protein 2 complex (AP-2) (Rappoport and Simon, 2009), which in turn recruits large amounts of clathrin, resulting in receptor clustering and rapid expansion of the budding vesicle (Sorkin et al., 1996; Tomas et al., 2014; Robinson, 2015). Accumulation of receptors in the bud further enhances cross-phosphorylation initiated by ligand binding (Ibach et al., 2015), thereby amplifying low-intensity signals. At the same time, phosphorylation of the β2 subunit of AP-2 by EGFR helps to initiate internalization, directing EGFR into the endocytic pathway (Fingerhut et al., 2001; Huang et al., 2003; Traub, 2009). The resulting endosomes dwell in the peripheral cytoplasm (Fig. 1, step 3A); here, maturation towards the late compartment is 'slow', and numerous recycling pathways are available to spare receptors from the degradation (Watanabe and Boucrot, 2017) that takes place in the perinuclear region of the cell, where proteolytic lysosomes abound (Johnson et al., 2016). While, at first, EGFR was considered to predominantly transduce signals at the plasma membrane, recent studies have shown that receptor endocytosis does not interfere with its signaling capabilities (Vieira et al., 1996; Sousa et al., 2012; Conte and Sigismund, 2016). Interestingly, it appears that for certain signaling pathways, such as activation of ERK1/2 proteins (also known as MAPK3 and MAPK1, respectively) downstream of EGFR, intracellular localization of signal transduction (i.e. at the plasma membrane versus on endosomes) correlates to the resulting transcriptional response (Sousa et al., 2012; Wu et al., 2012). In this way, spatial compartmentalization of signaling complexes fine-tunes their biological outcomes.

Fast and furious with ubiquitin

When the canonical endocytic route described above is saturated owing to increasing abundance of ligand, 'fast' clathrin-independent endocytosis (CIE) can take over, rapidly routing receptors toward degradation (Sigismund et al., 2005) (Fig. 1, step 3B). The decision to rapidly traffic endosomes carrying activated EGFR for degradation appears to be triggered by receptor ubiquitylation, as ubiquitylation-impaired EGFR overwhelmingly travels through the recycling-promoting CME route (Sigismund et al., 2005). Ubiquitylation of EGFR is mediated by the E3 ubiquitin ligase Cbl (Huang et al., 2006), which is targeted to the phosphorylated EGFR by the adaptor growth factor receptor-bound protein 2 (Grb2) (Batzer et al., 1994; Levkowitz et al., 1999; Jiang et al., 2003). Once ubiquitylated, EGFR can be recognized by the ubiquitin-dependent adaptors of the endosomal sorting complexes required for transport (ESCRTs) and sequestered into the intraluminal vesicles (ILVs) of the multivesicular body (MVB) (Henne et al., 2011). This physically removes the signaling tail of EGFR from the cytosol, effectively terminating the downstream signaling cascade (Eden et al., 2009). Receptor ubiquitylation exhibits a sigmoidal response to increasing concentrations of EGF, ensuring that under conditions of low ligand availability, activated EGFR will not be marked for destruction (Sigismund et al., 2013). Precisely what sets up this barrier to degradation is not entirely clear. One suggested mechanism postulates that high levels of receptor phosphorylation trigger simultaneous recruitment of Grb2 and Cbl2, resulting in efficient ubiquitylation (Sigismund et al., 2013). It is thought that a productive association of Cbl with the receptor is achieved above a certain threshold of phosphorylation, which couples ubiquitylation to the intensity of ligand-induced stimulus. In contrast, lower levels of stimulus offer fewer phosphorylated binding sites that are preferentially occupied by signaling molecules, such as Ras and phospholipase C (PLC)γ (Chardin et al., 1993; Haugh et al., 1999; Henriksen et al., 2013; Sigismund et al., 2013; Tomas et al., 2014). Thus, by segregating peripheral signaling and recycling pathways from perinuclear degradation in accordance with the degree of stimulation, cells can maximize life-sustaining inputs and effectively cope with overstimulation.

Journal of Cell Science (2017) 130, 4087-4096 doi:10.1242/jcs.209197

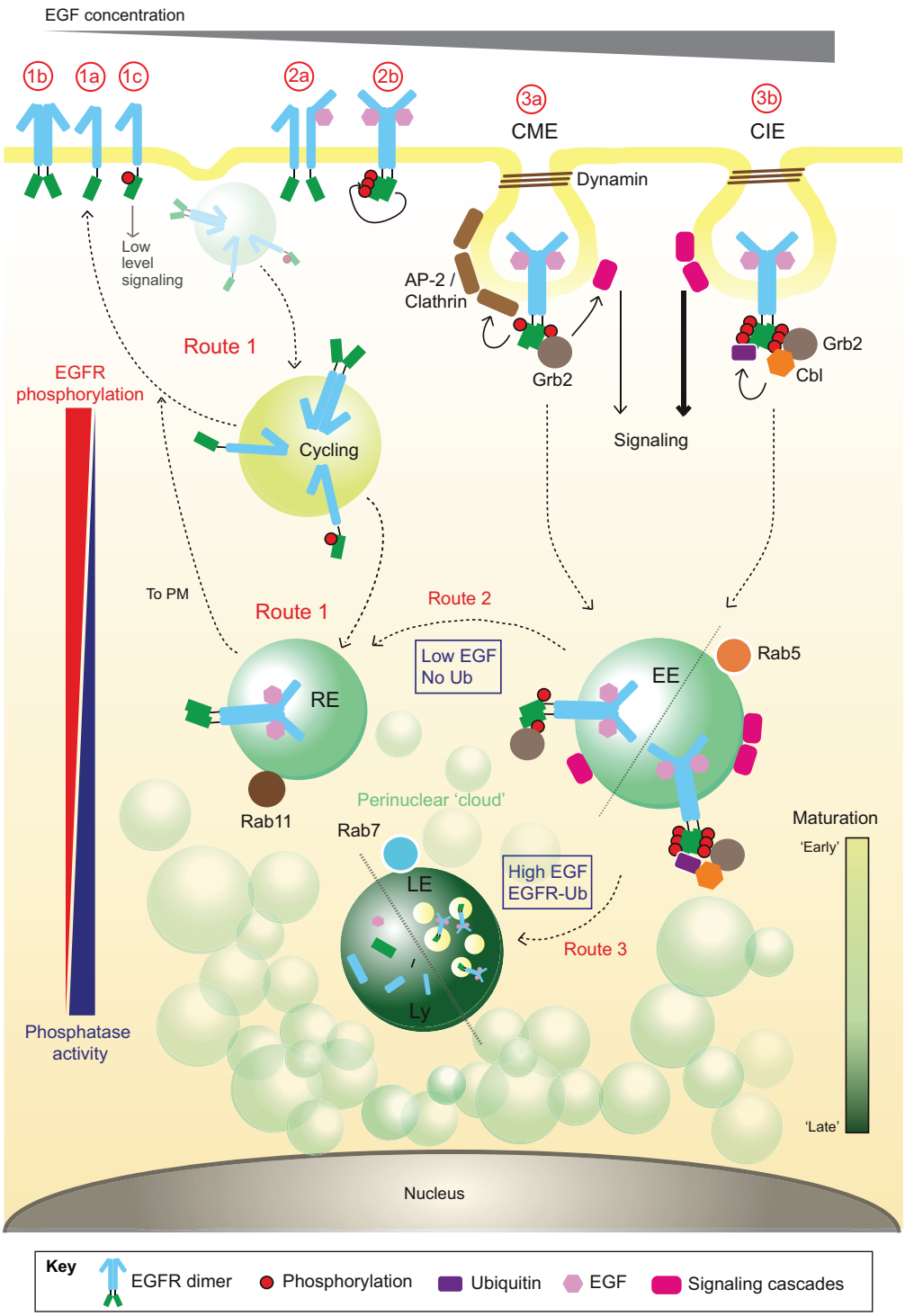

Fig. 1. Destinations of activated EGFR – from the cell periphery to the perinuclear 'cloud'. Ligand-free monomers of EGFR, residing primarily on the cell membrane (1a), can be spontaneously internalized and recycled (Route 1). Even in the absence of stimulation, stochastic dimerization (1b) and auto-activation (1c) of EGFR may occur. The latter is kept in check by endocytosis, inactivation and recycling through the Rab11 recycling endosomes (REs) (Route 1). Ligand binding promotes receptor dimerization (2a, 2b), leading to activation and phosphorylation of the cytoplasmic tails (2b) that mediate recruitment of various adaptor proteins (such as Grb2) (3a, 3b) for downstream signal transduction cascades. The intracellular fate of EGFR depends on the extent of its activation. Under conditions of 'low' stimulation, the AP-2 adaptor is recruited for clathrin-mediated endocytosis (CME) (3a), resulting in EGFR-containing early Rab5-positive signaling endosomes (3a, 3b). As these endosomes mature, they travel to the perinuclear region, where ligand-activated (and auto-activated) EGFR encounters increasing phosphatase activity and is inactivated prior to being recycled (Route 2). By contrast, 'high' levels of EGFR activation result in extensive receptor phosphorylation and ubiquitylation by the E3 ligase Cbl, which causes diversion of EGFR, which in this case is preferentially internalized by clathrin-independent endocytosis (CIE), away from recycling and towards degradation in the lysosome (Ly) located in the perinuclear 'cloud'. This occurs via the Rab7-positive late endosome (LE), where ubiquitylated EGFR is directed from the limiting endosomal membrane into intraluminal vesicles (ILVs), giving rise to a multivesicular body (MVB) (Route 3). Subsequent late endosome–lysosome fusion delivers EGFR for degradation.

Recycling goes deep

Receptors that are only moderately activated, either owing to low ligand availability or because they are activated in a ligand-independent manner, are still internalized into endosomes, but their reduced signaling potential does not require degradation. Upon entry into the early endosomal compartment, these receptors are recycled in vesicles characterized by the presence of the GTPase Rab11 (Ullrich et al., 1996; Baumdick et al., 2015). This pathway takes receptors through the perinuclear region, where they become increasingly exposed to the tyrosine-protein phosphatase non-receptor 1 (PTP1B; also known as PTPN1) that resides at the ER. PTP1B dephosphorylates EGFR at ER–endosome contact sites, ensuring that receptors transported back to the plasma membrane are no longer active. This mode of regulation results in an inverse spatial relationship between cellular kinase (peripheral) and phosphatase (perinuclear) activities (Fig. 1), which are facilitated by the interactions between endosomes and the ER (as discussed below). In contrast, fully activated EGFR molecules are redirected away from recycling vesicles and traffic toward the late endosomal compartment for degradation (Sabet et al., 2015). Prior to their

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degradation, these molecules also encounter the ER-associated phosphatase PTP1B (Eden et al., 2012b), which disables further signaling downstream. Additionally, in response to the intensity of incoming signals, the cell varies the number of signaling vesicles, which helps to maintain a relatively consistent amount of activated EGFR molecules per endosome (Villasenor et al., 2015). This, in turn, keeps the dephosphorylation rate constant and enables the cell to maintain robust responses to the dynamic extracellular environment without becoming vulnerable to overstimulation. The existence of multiple regulated means to abrogate signaling responses (i.e. dephosphorylation and degradation) underscores both the flexibility and rigor of the systems that function to keep cellular signaling cascades in check. Moreover, this complex regulatory framework exemplifies how spatiotemporal regulatory capabilities of the endocytic compartment elegantly serve the greater interests of the cell. How the trafficking and transport of EGFR is orchestrated in molecular terms is discussed in the next section.

Cruising in the endosome – how mature!

Ready, set, phosphoinositides

Reversible association of proteins and complexes with specific vesicular membranes underlies the membrane dynamics throughout the endosomal system. To ensure recruitment and exclusion at the right place and time, vesicles undergo continuous maturation, with their different stages characterized by the presence of distinct phosphoinositides (PIs). These derivatives of phosphatidylinositol are anchored to the membrane and acquire different phosphorylation states, which then direct the differential recruitment of proteins associated with early or late stages of endosomal maturation (reviewed by Schink et al., 2016). Not surprisingly then, progress of EGFR along the endocytic route closely depends on the PI contents of its carrier vesicles (Tan et al., 2015; Schink et al., 2016; Henmi et al., 2016). In fact, activated EGFR can itself influence membrane composition through the recruitment of phosphoinositide 3-kinase (PI3K) Iiα (also known as PIK3R1), which increases the concentration of phosphatidylinositol 3-phosphate [PI(3)P]. The presence of this lipid, in turn, stimulates the recruitment of the small GTPase Rab5 (predominantly the Rab5a isoform) – the central organizer of early endosomes (Zerial and McBride, 2001; Jordens et al., 2005; Zeigerer et al., 2012) – and thus marks the start of endosomal maturation (Leevers et al., 1999; Ceresa and Peterson, 2014). Therefore, by manipulating membrane features, EGFR effectively accelerates its own trafficking and downregulation.

Rab5 is on

Once EGFR, residing on the surface of the cell, turns ‘on’ and moves into newly budding vesicles, it sets in motion an orderly chain of arrivals and departures of membrane-associated proteins that facilitate and control its progress along the endocytic track. This begins with recruitment of factors responsible for the establishment of early endosomal character, marked by the presence of the Rab5 GTPase. Firstly, the guanine nucleotide exchange factor (GEF) RME-6, which activates the Rab5 GTPase, associates with the budding membrane to promote Rab5 recruitment towards the nascent endosome (Sato et al., 2005) (Fig. 2, step 1A). After the EGFR-containing endosome buds off to begin its intracellular journey, it acquires another Rab5 GEF, Rin-1 (Balaji et al., 2012) (Fig. 2, step 1B). This likely leads to increased levels of Cbl associated with EGFR and, consequently, stimulates receptor ubiquitylation (Barbieri et al., 2004). Ubiquitylated EGFR, in turn, recruits yet another Rab5 GEF, Rabex-5 (also known as RABGEF1) (Fig. 2, step 1C). Collectively, these steps create a positive-feedback loop of GTP-loaded (and thus active) Rab5 membrane occupancy (Penengo et al., 2006; Mattera et al., 2006; Zhang et al., 2014), thereby stabilizing Rab5-associated machineries that are responsible for early endosome fusion and transport. Specifically, tethering factor EEA1 is recruited to Rab5 (Simonsen et al., 1998; Dumas et al., 2001; Navaroli et al., 2012), which, together with the class C core vacuole/endosome tethering (CORVET) complex, promotes fusion between early endosomes (Balderhaar et al., 2013; Van der Kant et al., 2015) (Fig. 2, step 2). At the same time, Rab5-positive endosomes move away from the plasma membrane towards the perinuclear region, where their fusion with later-stage endosomes is more likely. This transport is accomplished by the minus-end-directed dynein motor complex, which is linked to Rab5 through its effector FHIP [for ‘Fused TOES (FTS)-Hook-FTS and HOOK-interacting protein’; also known as FHF and FAM160A2] (Driskell et al., 2007; Guo et al., 2016) (Fig. 2, step 3). Taken together, the processes orchestrated by the Rab5 GTPase enable early endosomes to grow in size, in preparation for their transition into the late compartment, where cargo proteolysis occurs.

Hand it over to Rab7

Late endosomal vesicles are typically marked by the GTPase Rab7 (which has Rab7a and Rab7b forms) and devoid of Rab5. Occurring through an elegant hand-over mechanism, the conversion from Rab5- into Rab7-labeled vesicles is the hallmark of endosomal maturation (Pols et al., 2013; Balderhaar et al., 2013; Lin et al., 2014; van der Kant et al., 2015; McEwan et al., 2015) (Fig. 2, step 4). This begins with the arrival of the Mon1–Ccz1 complex (Fig. 2, step 4A), which interacts with both Rab5 and Rabex-5 (Poteryaev et al., 2010; Nordmann et al., 2010; Huotari and Helenius, 2011). Subsequent dephosphorylation of PI3P on the endosomal membrane (Shinde and Maddika, 2016) enables Mon1–Ccz1 to attract and activate Rab7 (by loading it with GTP; Fig. 2, step 4C) (Nordmann et al., 2010; Yasuda et al., 2016), as well as to displace Rabex-5 (Fig. 2, step 4B) (Rink et al., 2005), resulting in a hybrid vesicle harboring both Rab5 and Rab7. At this point, the GTPase-activating protein (GAP) Msb3 can be recruited to expel Rab5 from the endosomal membrane (Fig. 2, step 4D) (John Peter et al., 2013). Finally, interaction of the GAP TBC-2 with PI(3)P stimulates the removal of Rab5 from the maturing endosomal membrane (Fig. 2, step 4E) (Law et al., 2017). Taken together, this interconnected cascade of molecular events organizes the conversion of a Rab5-positive early endosome into a later one marked by Rab7 (John Peter et al., 2013; Rana et al., 2015). Owing to the presence of Rab7, the late endosome can now acquire the dynein motor machinery via the Rab7 effector protein Rab-interacting lysosomal protein (RILP) and move along microtubules towards the perinuclear region (Cantalupo et al., 2001; Jordens et al., 2001) (Fig. 2, step 5). This Rab7-associated transport complex also recruits the homotypic fusion and vacuolar protein-sorting (HOPS) complex, effectively coupling minus-end-directed transport to fusion of late endosomes with one another or with lysosomes carrying Rab7/HOPS (Ungermann et al., 2000; Van der Kant et al., 2015). Along their journey, late endosomes receive key inputs and direction from the ER, and we discuss our current understanding of this below.

Here comes the ER for a meet-and-greet

Endosomes in the cloud

Once early endosomes begin to mature, they are increasingly guided by interactions with the ER (Friedman et al., 2013). Transient

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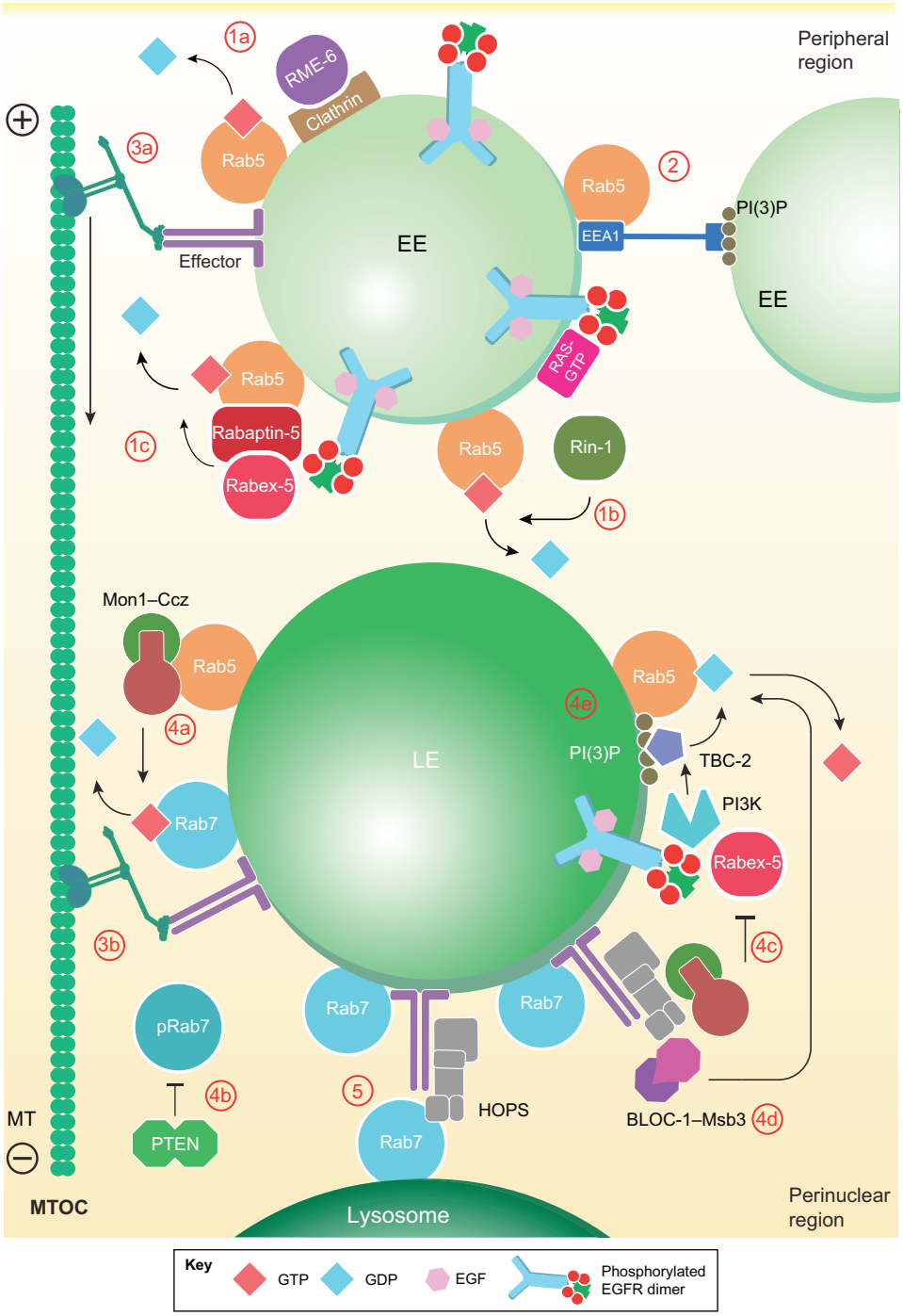

Fig. 2. The order of molecular events in the maturation and transport of EGFR-containing endosomes. Following endocytosis of activated EGFR, early endosomes (EEs) acquire the GTPase Rab5, which is activated by its GEFs RME-6 (1a) and Rin-1 (1b). Meanwhile, ubiquitylated EGFR recruits the Rab5 effector Rabaptin-5 and another Rab5 GEF, Rabex-5 (1c). Once stably associated with the endosomal membrane, Rab5 can recruit its effector proteins EEA1 and FHIP, which respectively mediate early fusion events (2) and transport (3) along microtubule (MT) tracks, carried out by the dynein motor complex toward the nucleus (the minus-end of microtubules). As EEs mature into late endosomes (LEs), they acquire the GTPase Rab7 and 'kick' off Rab5 (4). First, Rab5 recruits the Rab7 GEF complex, Mon1–Ccz1 (4a), which activates Rab7, resulting in a hybrid Rab5 and Rab7 endosome. Mon1–Ccz1 also displaces Rabex-5 (4b). Recruitment of Rab7 is further modulated through the dephosphorylation activity of PTEN (4c). To complete the Rab5-to-Rab7 handover, the Rab5 GAPs Msb3 (via the BLOC-1 complex) (4d) and TBC-2 (4e) associate with Rab7 to promote inactivation and release of Rab5. Through its effector RILP, Rab7 can recruit the dynein motor for minus-end-directed transport (5) and the HOPS tethering complex for fusion (6), thereby coupling late endosome transport towards and fusion with the lysosome in order to efficiently deliver activated EGFR for degradation. MTOC, microtubule-organizing center.

physical contacts between these two organelles coordinate long-range vesicle transport, regulate membrane dynamics within the maturing endosome and influence the receptor signaling status (Eden et al., 2012b). In the fast-paced world of endosomal flux, knowing where and when to go is crucial (Neefjes et al., 2017). To achieve this task, cells partition their endosomal compartment into two fractions – a motile peripheral pool of vesicles and a comparatively stationary perinuclear 'cloud' of endosomes that is located around the Golgi complex (Jongsma et al., 2016). This organization is critical for endosomes to efficiently meet each other and mature. The perinuclear endosomal pool is kept in place by the ER-located ubiquitin ligase RNF26 (Fig. 3, step 1) (Jongsma et al., 2016), which recruits and ubiquitylates SQSTM1 (also known as p62), a protein best known for its function as an autophagy adaptor. The resulting complex is able to position specific endosomes at the ER by attracting EPS15, which is present on the earliest vesicles, and TOLLIP, located on later endosomes, through their ubiquitin-binding domains. When endosomes need to leave the cloud, the deubiquitylating enzyme USP15 releases them from the 'grip' of the ER, allowing their long-range transport (Jongsma et al., 2016). Inhibition of this positioning mechanism dislocates the entire endosomal system, which results in the failure of endosomes to progressively mature, attenuates cargo degradation and leads to continued EGFR signaling (Jongsma et al., 2016). Consequently,

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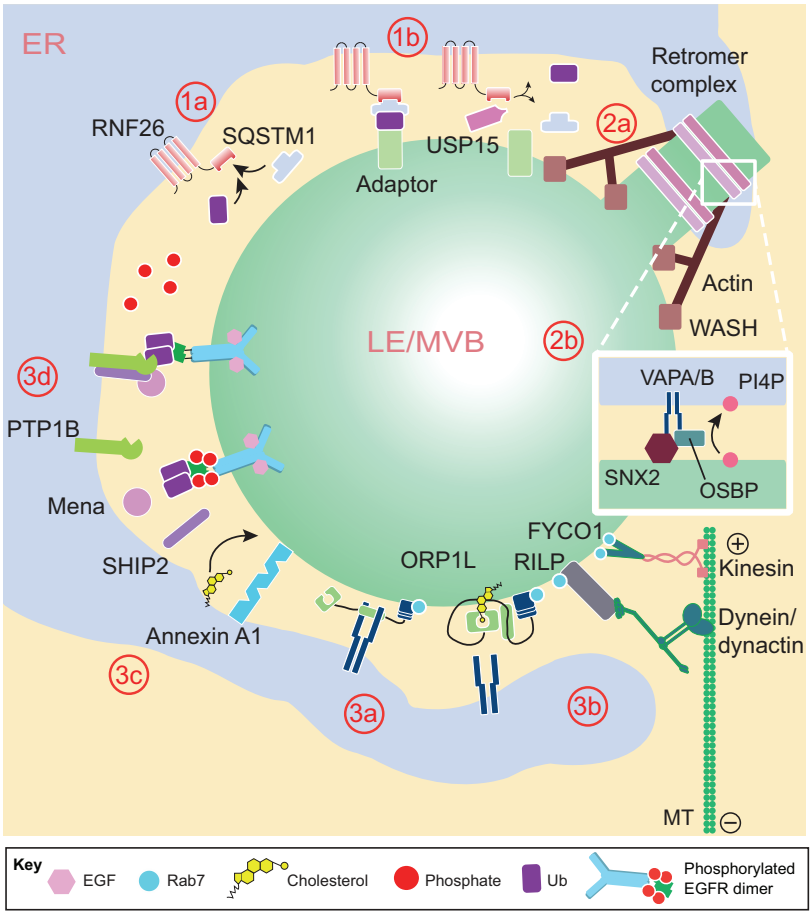

Fig. 3. ER-mediated regulation of the EGFR-containing endosome. When challenged with ligand, EGFR-containing endosomes travel from the cell periphery to the perinuclear vesicle 'cloud', where their maturation and degradation of activated receptors occur. (1) The perinuclear cloud is regulated by the ER-located E3 ligase RNF26, which recruits and ubiquitylates SQSTM1 (1a). The resulting ER-associated complex then positions endosomes by attracting various ubiquitin-binding endosomal adaptors. Deubiquitylation of SQSTM1 by the DUB USP15 can release positioned endosomes for continued transport (1b). (2) Maturation of endosomes requires them to expel cargoes not intended for degradation. This recycling process is supported by the ER, where the ER-bound proteins VAPA and VAPB interact with the retromer complex subunit SNX2 (2a). At this ER–endosome contact site, the WASH complex induces local actin polymerization (2b) to promote fission of recycling tubules away from the maturing endosome. (3) The maturing endosome travels toward the lysosome. This transport is mediated by the Rab7–RILP–dynein motor complex, and is controlled by the cholesterol sensor ORP1L. When cholesterol is abundant in the endosomal membrane, minus-end transport is uninhibited. By contrast, if cholesterol is depleted, ORP1L can interact with VAPA, resulting in release of the dynein motor (3a). At this juncture, facilitated by the ER-associated protrudin, Rab7 may be able to switch direction of transport by acquiring the effector FYCO1 and the kinesin-1 motor (3b). At the ORP1L/VAP ER–endosome contact site, the annexin 1A tether mediates cholesterol transfer from the ER to the endosome (3c), which promotes incorporation of EGFR into the ILVs for degradation. Prior to targeting of EGFR to ILVs, activated receptor is dephosphorylated by the phosphatase PTP1B, with the help of the phosphatase SHIP2 and actin-nucleating protein Mena (3d).

through the activity of RNF26, the ER promotes trafficking of activated EGFR and enables timely termination of its signaling.

ER goes in for a hug

EGFR-containing endosomes that travel toward the lysosome must expel any cargoes that are not destined for degradation. This type of recycling intimately involves the ER (Fig. 3, step 2). To this end, the retromer complex subunit SNX2 that is bound to PI3P on endosomal membrane interacts with the vesicle-associated protein A/B (VAPA/B) on the ER membrane; the resulting complex couples recycling tubule formation with the transient WASH-mediated assembly of a localized actin cytoskeleton, which is required for fission (Dong et al., 2016). In effect, this 'embrace' of the recycling tubule by the ER dictates both the exact location and timing of fission (Rowland et al., 2014).

As soon as maturing endosomes acquire Rab7, they begin to contact the ER for guidance on directionality of their transport throughout the cell. As mentioned above, Rab7 mediates dynein-dependent transport of late endosomes toward the nucleus through its effector RILP (Jordens et al., 2001), which is needed to bring late endosome cargo, such as the EGFR, to the lysosome (Driskell et al., 2007). However, Rab7 can also 'choose' to recruit the effector FYCO1 and, subsequently, the kinesin-1 motor, which enables microtubule-based transport of late endosomes in the opposite (plus-end) direction (i.e. toward the periphery of the cell) (Pankiv et al., 2010). In order for Rab7 to mediate a course change from one direction to the other, it needs to disengage from one motor complex, while recruiting another. Interestingly, both release of the dynein motor and acquisition of kinesin-1 involve help from the ER. To achieve the former, Rab7 interacts with the cholesterol sensor Rab7-associated oxysterol-binding protein (ORP1L; also known as OSBPL1A) (Fig. 3, step 3) (Rocha et al., 2009). As long as endosomal cholesterol is available, ORP1L remains in a closed conformation, which is compatible with maintenance of the interaction between the dynein transport complex and Rab7–RILP. Conversely, under conditions of cholesterol depletion from the endosomal membrane, ORP1L opens up to interact with the ER-bound VAPA/B (Rocha et al., 2009; Van der Kant et al., 2013; Wijdeven et al., 2016). This results in release of dynein from the Rab7–RILP complex and temporarily halts transport of the endosome toward the microtubule minus-end. Incidentally, VAPA/B also interacts with the ER-associated protein protrudin, which is capable of loading kinesin-1 motor onto Rab7–FYCO1 (Raiborg et al., 2015). It has been speculated that this scenario presents an opportunity for Rab7 to switch the direction of endosomal transport away from the nucleus (Wijdeven et al., 2015; Raiborg et al., 2016). Although EGFR-containing late endosomes have not been shown to travel via this plus-end-directed route, whether and how Rab7, or its associated proteins, may 'guard' against the misdirection of EGFR is an important issue that remains largely unexplored.

In addition to modulating endosomal transport, ER–endosome contact sites established by the ORP1L–VAPA/B interaction allow endosomal cholesterol to be replenished directly from the ER. The first steps of EGFR signal inactivation also take place at ER–endosome contact sites, this time established by way of an annexin 1A tether (Eden et al., 2016). Meanwhile, the first steps of signal inactivation also take place at ER–endosome contact sites. It is here

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that EGFR encounters the phosphatase PTP1B, which resides on the ER membrane, and the subsequent dephosphorylation of its cytoplasmic tail renders the receptor inactive (Eden et al., 2012b). Interaction between phosphorylated EGFR and PTP1B is likely regulated by the two adaptor proteins Mena (also known as Enah) and Ship (also known as Inpp5d) (Hughes et al., 2015). Both receptor dephosphorylation and replenishment of late-endosomal cholesterol promote the incorporation of EGFR into the ILVs of a maturing MVB (Raiborg and Stenmark, 2009; Eden et al., 2010, 2012b), which physically removes the tail of EGFR from the cytosol, effectively terminating its signaling. This spatially and temporally links receptor inactivation to its degradation. The details of how EGFR finds its way inside the MVB are discussed below.

The final act – inactivation and destruction

In the final throes of the life of EGFR, late endosomes that arrive in the perinuclear region of the cell fuse with the proteolytic lysosome stationed here (Luzio et al., 2007; Johnson et al., 2016). To get into the lysosome, ubiquitylated EGFR molecules are escorted to the site of ILV formation by four sequentially operating ESCRT complexes, ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III (Christ et al., 2017)

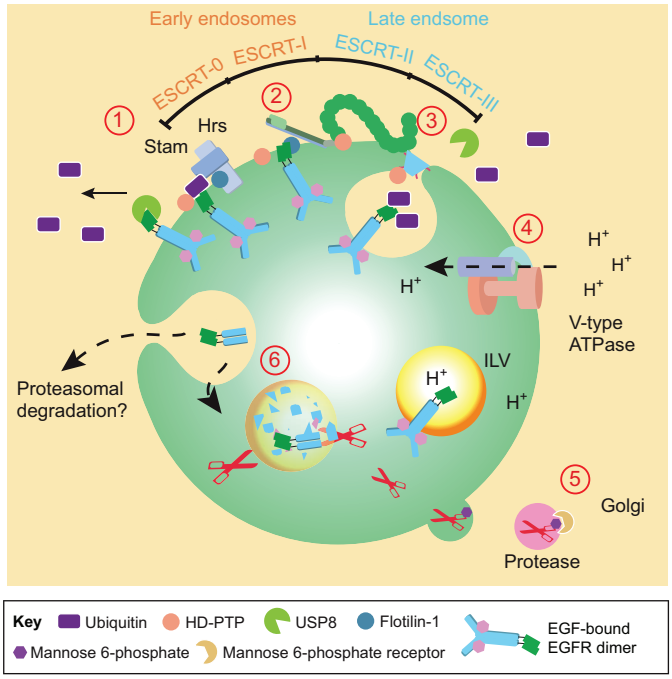

Fig. 4. In or out – sorting and degradation of EGFR. Degradation of activated EGFR necessitates its sorting and incorporation into the ILVs of a maturing MVB, which are orchestrated by the ESCRT system. This begins on early endosomes, where the ESCRT-0 complex, consisting of the ubiquitin-binding adaptor proteins Hrs and STAM, sorts ubiquitylated EGFR to the MVB (1). Assisted by flotilin-1, EGFR is subsequently transferred to the ESCRTs -I, -II, and -III (2). ESCRT-III deforms the limiting membrane of the MVB, resulting in ILV formation and sequestration of EGFR therein. Deubiquitylation of EGFR by the ESCRT-0-associated DUB USP8 can spare the receptor from degradation. USP8 can also interact with ESCRT-III and, in the presence of the phosphatase HD-PTP, may promote transfer of EGFR down the ESCRT pathway. (3) Proteolytic capabilities of late endosomes and lysosomes require an acidic environment as provided by V-type ATPases (4); this is optimal for denaturation and degradation by the lysosomal proteases, which are transported by the mannose 6-phosphate receptor from the Golgi (5). While the transmembrane section of EGFR is thought to be degraded by the rhomboid proteases, how – and whether – the cytoplasmic tail of EGFR is degraded remains unclear (6).

(Fig. 4). In a first selection step, taking place on early endosomes, the ESCRT-0 complex, which comprises the hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs, also known as Hgs) and signal transduced adaptor molecule (STAM), recognizes and sequesters ubiquitylated EGFR away from recycling domains. Interestingly, STAM and Hrs are both phosphorylated by EGFR, following its kinase domain activation, and dephosphorylated by PTP1B (Eden et al., 2010; Stuible and Tremblay, 2010). Co-regulation of ESCRT-0 with the EGFR activity cycle temporally synchronizes peak sorting activity, with sharply increasing demand following ligand-mediated receptor activation. Once ubiquitylated, EGFR traffics to the late endosome, and ESCRT-I, -II and -III are sequentially recruited to sort and package the chosen cargoes into ILVs. In conjunction with flotillin-1, ESCRT-I transfers ubiquitylated receptors to ESCRT-II, which results in the accumulation of degradation substrates, invagination of the limiting endosomal membrane and ESCRT-III-dependent formation of ILVs (Meister et al., 2017; Christ et al., 2017). EGFR can escape ubiquitin-dependent sorting into ILVs, either early on in the endosomal pathway through deubiquitylation by the STAM-associated deubiquitylating enzyme (DUB) USP8 (also referred to as UBPY) (Niendorf et al., 2007; Berlin et al., 2010), or at the limiting membrane of the MVB (Eden et al., 2012a). In addition to binding to ESCRT-0, USP8 also interacts with ESCRT-III components further down the sorting pathway (Row et al., 2007), and a recent report suggests that USP8 can promote the switch between ESCRT complexes on the EGFR substrate through an ESCRT-0 accessory protein HD-PTP (also known as PTPN23) (Ali et al., 2013). Ubiquitylation not only controls the fate of cargoes such as EGFR, but also regulates the function of ESCRT proteins themselves. For instance, the oncogene LAPTM4B promotes the ubiquitylation of Hrs by the E3 ligase NEDD4, which renders this adaptor unable to recognize ubiquitylated receptors (Hoeller et al., 2006; Persaud et al., 2009; Tan et al., 2015). By contrast, the accumulation of phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2] and the resulting recruitment of SNX5 to the endosomal membrane inhibits Hrs ubiquitylation and instead promotes recognition of ubiquitylated cargoes by the ESCRT-0 complex (Tan et al., 2015). Because PI exchange on the endosomal membrane coincides with maturation, this the above regulatory module couples sorting of EGFRs that are marked for destruction to the physical progression of receptor-containing vesicles along the endocytic route.

Finally, to complete its life cycle, EGFR must be delivered to the lysosome. To accomplish this, the MVB must fuse with the lysosome, depositing its ILVs into the proteolytic lysosomal lumen (Luzio et al., 2007). Here, the luminal part of EGFR (i.e. its ligand-binding domain) is degraded after an unfolding step, which likely first requires the reduction of the cysteine bridges by the protein GILT (also known as IFI30) (Arunachalam et al., 2000), followed by the action of multiple glycosidases and proteases of the cathepsin family. Furthermore, the transmembrane domain of EGFR is cleaved by the transmembrane aspartate proteases of the rhomboid family (Lemberg and Freeman, 2007). However, the fate of the remaining cytoplasmic tail remains unclear. While it has been postulated that the tail may be expelled in the cytosol, experimental demonstration of this is yet to be reported. Although the pathway towards degradation of EGFR is at this time fairly clear, the mechanisms of its actual destruction are much less understood.

Conclusions and perspectives

At the time of writing, a PubMed search for the term ‘EGFR’ returned over 44,000 publications, of which the vast majority

primarily consider the immediate steps in the life of an activated receptor – those occurring at the cell surface. However, an EGFR molecule that has been turned 'on' likely spends more time traveling the endosomal system than residing at the cell surface. Meanwhile, its cytoplasmic tail remains exposed and available for signaling. Interestingly, the quality of signaling may be different in the cell interior than at the plasma membrane. However, due to the transient nature of endosomes and their ability to move swiftly through the cell, it has been challenging to understand what happens to EGFR on this complex journey, and when. Recent advances in imaging tools and techniques have enabled us to make substantial progress in addressing these questions and have revealed the intricate molecular mechanisms at play, as well as the subtle ways in which EGFR influences them to promote its own demise. As it moves in endosomes towards the perinuclear cloud, en route to its final destination in the lysosome, active EGFR is subjected to regulation by the ER at the ER–endosome contact sites. As EGFR, now marked for destruction with ubiquitin, reaches the MVB, termination of its signaling is ensured by dephosphorylation and subsequent inclusion into the ILVs. But what if the receptor thus committed could escape the ILVs back to the limiting membrane of the MVB? Could its signaling from endosomes resume? Or would the receptor still be recycled and reused at the cell surface? Perhaps these provocative questions will find answers in the next phase of the discovery regarding endocytosis and the management of key cellular cargoes, with EGFR at their forefront.

Competing interests
The authors declare no competing or financial interests.

Funding
Our work in this area was supported by the Institute of Chemical Immunology, an NWO Gravitation project funded by the Ministry of Education, Culture and Science of the government of the Netherlands and an European Research Council Advanced Grant to J.N.

References
Ali, N., Zhang, L., Taylor, S., Mironov, A., Urbé, S. and Woodman, P. (2013). Recruitment of UBPY and ESCRT exchange drive HD-PTP-dependent sorting of EGFR to the MVB. *Curr. Biol.* **23**, 453-461.

Arunachalam, B., Phan, U. T., Geuze, H. J. and Cresswell, P. (2000). Enzymatic reduction of disulfide bonds in lysosomes: characterization of a gamma-interferon-inducible lysosomal thiol reductase (GILT). *Proc. Natl. Acad. Sci. USA* **97**, 745-750.

Azimzadeh Irani, M., Kannan, S. and Verma, C. (2017). Role of N-glycosylation in EGFR ectodomain ligand binding. *Proteins* **85**, 1529-1549.

Bajaj, M., Waterfield, M. D., Schlessinger, J., Taylor, W. R. and Blundell, T. (1987). On the tertiary structure of the extracellular domains of the epidermal growth factor and insulin receptors. *Biochim. Biophys. Acta* **916**, 220-226.

Balaji, K., Mooser, C., Janson, C. M., Bliss, J. M., Hojjat, H. and Colicelli, J. (2012). RIN1 orchestrates the activation of RAB5 GTPases and ABL tyrosine kinases to determine the fate of EGFR. *J. Cell Sci.* **125**, 5887-5896.

Balderhaar, H. J. K., Lachman, J., Yavavli, E., Brocker, C., Lurick, A. and Ungermann, C. (2013). The CORVET complex promotes tethering and fusion of Rab5/Vps21-positive membranes. *Proc. Natl. Acad. Sci. USA* **110**, 3823-3828.

Barbieri, M. A., Fernandez-Pol, S., Hunker, C., Horazdovsky, B. H. and Stahl, P. D. (2004). Role of rab5 in EGF receptor-mediated signal transduction. *Eur. J. Cell Biol.* **83**, 305-314.

Batzer, A. G., Rotin, D., Ureña, J. M., Skolnik, E. Y. and Schlessinger, J. (1994). Hierarchy of binding sites for Grb2 and Shc on the epidermal growth factor receptor. *Mol. Cell. Biol.* **14**, 5192-5201.

Baumdick, M., Bruggemann, Y., Schmick, M., Xouri, G., Sabet, O., Davis, L., Chin, J. W., Bastiaens, P. I. H. (2015). EGF-dependent re-routing of vesicular recycling switches spontaneous phosphorylation suppression to EGFR signaling. *Elife* **4**, e12223.

Berlin, I., Schwartz, H. and Nash, P. D. (2010). Regulation of epidermal growth factor receptor ubiquitination and trafficking by the USP8·STAM complex. *J. Biol. Chem.* **285**, 34909-34921.

Brand, T. M., Iida, M., Luthar, N., Starr, M. M., Huppert, E. J. and Wheeler, D. L. (2013). Nuclear EGFR as a molecular target in cancer. *Radiother. Oncol.* **108**, 370-377.

Bublil, E. M. and Yarden, Y. (2007). The EGF receptor family: spearheading a merger of signaling and therapeutics. *Curr. Opin. Cell Biol.* **19**, 124-134.

Burgess, A. W., Cho, H.-S., Eigenbrot, C., Ferguson, K. M., Garrett, T. P. J., Leahy, D. J., Lemon, M. A., Sliwkowski, M. X., Ward, C. W. and Yokoyama, S. (2003). An open-and-shut case? Recent Insights into the Activation of EGF/ErbB Receptors. *Mol. Cell* **12**, 514-552.

Burke, P. M. and Wiley, H. S. (1999). Human mammary epithelial cells rapidly exchange empty EGFR between surface and intracellular pools. *J. Cell. Physiol.* **180**, 448-460.

Cantalupo, G., Alifano, P., Roberti, V., Bruni, C. B. and Bucci, C. (2001). Rab-interacting lysosomal protein (RILP): the Rab7 effector required for transport to lysosomes. *EMBO J.* **20**, 683-693.

Ceresa, B. P. and Peterson, J. L. (2014). Cell and molecular biology of epidermal growth factor receptor. *Int. Rev. Cell Mol. Biol.* **313**, 145-178.

Chardin, P., Camonis, J. H., Gale, N. W., van Aelst, L., Schlessinger, J., Wigler, M. H. and Bar-Sagi, D. (1993). Human Sos1: a guanine nucleotide exchange factor for Ras that binds to Grb2. *Science* **260**, 1338-1343.

Chen, J., Zeng, F., Forrester, S. J., Eguchi, S., Zhang, M.-Z. and Harris, R. C. (2016). Expression and function of the epidermal growth factor receptor in physiology and disease. *Physiol. Rev.* **96**, 1025-1069.

Chen, R., Jin, G. and McIntyre, T. M. (2017). The soluble protease ADAMDEC1 released from activated platelets hydrolyzes platelet membrane pro-epidermal growth factor (EGF) to active high-molecular-weight EGF. *J. Biol. Chem.* **292**, 10112-10122.

Christ, L., Raiborg, C., Wenzel, E. M., Campsteijn, C. and Stenmark, H. (2017). Cellular functions and molecular mechanisms of the ESCRT membrane-scission machinery. *Trends Biochem. Sci.* **42**, 42-56.

Cohen, S. (1962). Isolation of. mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the new-born animal. *J. Biol. Chem.* **237**, 1555-1562.

Cohen, S. and Carpenter, G. (1975). Human epidermal growth factor: isolation and chemical and biological properties. *Proc. Natl. Acad. Sci. USA* **72**, 1317-1321.

Conte, A. and Sigismund, S. (2016). Chapter six - the ubiquitin network in the control of EGFR Endocytosis and Signaling. *Prog. Mol. Biol. Transl. Sci.* **141**, 225-276.

Dong, R., Saheki, Y., Swarup, S., Lucast, L., Harper, J. W. and De Camilli, P. (2016). Endosome-ER contacts control actin nucleation and retromer function through VAP-dependent regulation of PI4P. *Cell* **166**, 408-423.

Driskell, O. J., Mironov, A., Allan, V. J. and Woodman, P. G. (2007). Dynein is required for receptor sorting and the morphogenesis of early endosomes. *Nat. Cell Biol.* **9**, 113-120.

Dumas, J. J., Merithew, E., Sudharshan, E., Rajamani, D., Hayes, S., Lawe, D., Corvera, S. and Lambright, D. G. (2001). Multivalent endosome targeting by homodimeric EEA1. *Mol. Cell* **8**, 947-958.

Eden, E. R., White, I. J. and Futter, C. F. (2009). Down-regulation of epidermal growth factor receptor signalling within multivesicular bodies. *Biochem. Soc. Trans.* **37**, 173-177.

Eden, E. R., White, I. J., Tsapara, A. and Futter, C. E. (2010). Membrane contacts between endosomes and ER provide sites for PTP1B-epidermal growth factor receptor interaction. *Nat. Cell Biol.* **12**, 267-272.

Eden, E. R., Huang, F., Sorkin, A. and Futter, C. E. (2012a). The role of EGF receptor ubiquitination in regulating its intracellular traffic. *Traffic* **13**, 329-337.

Eden, E. R., Burgoyne, T., Edgar, J. R., Sorkin, A. and Futter, C. E. (2012b). The relationship between ER-multivesicular body membrane contacts and the ESCRT machinery. *Biochem. Soc. Trans.* **40**, 464-468.

Eden, E. R., Sanchez-Heras, E., Tsapara, A., Sobota, A. Levine, T. P. and Futter, C. E. (2016). Annexin A1 tethers membrane contact sites that mediate ER to endosome cholesterol transport. *Dev. Cell* **37**, 473-483.

Ferguson, K. M., Berger, M. B., Mendrola, J. M., Cho, H.-S., Leahy, D. H. and Lemmon, M. A. (2003). EGF activates its receptor by removing interactions that autoinhibit ectodomain dimerization. *Mol. Cell* **11**, 507-517.

Fingerhut, A., von Figura, K. and Höning, S. (2001). Binding of AP2 to sorting signals is modulated by AP2 phosphorylation. *J. Biol. Chem.* **276**, 5476-5482.

Foley, J., Nickerson, N., Riese, D. J., II, Hollenhorst, P. C., Lorch, G. and Foley, A. M. (2012). At the crossroads: EGFR and PTHrP signaling in cancer-mediated diseases of bone. *Odontology* **100**, 109-129.

Francavilla, C., Papetti, M., Rigbolt, K. T. G., Pedersen, A.-K., Sigurdsson, J. O., Cazzamali, G., Karemore, G., Blagoev, B. and Olsen, J. V. (2016). Multilayered proteomics reveals molecular switches dictating ligand-dependent EGFR trafficking. *Nat. Struct. Mol. Biol.* **23**, 608-618.

Friedman, J. R., Dibenedetto, J. R., West, M., Rowland, A. A. and Voeltz, G. K. (2013). Endoplasmic reticulum-endosome contact increases as endosomes traffic and mature. *Mol. Biol. Cell* **24**, 1030-1040.

Guo, X., Farías, G. G., Mattera, R. and Bonifacino, J. S. (2016). Rab5 and its effector FHF contribute to neuronal polarity through dynein-dependent retrieval of somatodendritic proteins from the axon. *Proc. Natl. Acad. Sci. USA* **113**, E5318-E5327.

Harris, R. C., Chung, E. and Coffey, R. J. (2003). EGF receptor ligands. *Exp. Cell Res.* **284**, 2-13.

Haugh, J. M., Huang, A. C., Wiley, H. S., Wells, A. and Lauffenburger, D. A. (1999). Internalized epidermal growth factor receptors participate in the activation of p21ras in fibroblasts. *J. Biol. Chem.* **274**, 34350-34360.
Henmi, Y., Morikawa, Y., Oe, N., Ikeda, N., Fujita, A., Takei, K., Minogue, S. and Tanabe, K. (2016). PtdIns4KIIα generates endosomal PtdIns(4)P and is required for receptor sorting at early endosomes. *Mol. Biol. Cell* **27**, 990-1001.
Henne, W. M., Buchkovich, N. J. and Emr, S. D. (2011). The ESCRT pathway. *Dev. Cell* **21**, 77-91.
Henriksen, L., Grandal, M. V., Knudsen, S. L. J., van Deurs, B. and Grøvdal, L. M. (2013). Internalization mechanisms of the epidermal growth factor receptor after activation with different ligands. *PLoS ONE* **8**, e58148.
Herbst, J. J., Opresko, L. K., Walsh, B. J., Lauffenburger, D. A. and Wiley, H. S. (1994). Regulation of postendocytic trafficking of the epidermal growth factor receptor through endosomal retention. *J. Biol. Chem.* **269**, 12865-12873.
Hoeller, D., Crosetto, N., Blagoev, B., Raiborg, C., Tikkanen, R., Wagner, S., Kowanetz, K., Breitling, R., Mann, M., Stenmark, H. et al. (2006). Regulation of ubiquitin-binding proteins by monoubiquitination. *Nat. Cell Biol.* **8**, 163-169.
Honegger, A. M., Kriss, R. M., Ullrich, A. and Schlessinger, J. (1989). Evidence that autophosphorylation of solubilized receptors for epidermal growth factor is mediated by intramolecular cross-phosphorylation. *Proc. Natl. Acad. Sci. USA* **86**, 925-929.
Huang, F., Jiang, X. and Sorkin, A. (2003). Tyrosine phosphorylation of the beta2 subunit of clathrin adaptor complex AP-2 reveals the role of a di-leucine motif in the epidermal growth factor receptor trafficking. *J. Biol. Chem.* **278**, 43411-43417.
Huang, F., Kirkpatrick, D., Jiang, X., Gygi, S. and Sorkin, A. (2006). Differential regulation of EGF receptor internalization and degradation by multiubiquitination within the kinase domain. *Mol. Cell* **21**, 737-748.
Hughes, S. K., Oudin, M. J., Tadros, J., Neil, J., Del Rosario, A., Joughin, B. A., Ritsma, L., Wyckoff, J., Vasile, E., Eddy, R. et al. (2015). PTP1b-dependent regulation of receptor tyrosine kinase signaling by the actin-binding protein Mena. *Mol. Biol. Cell* **26**, 3867-3878.
Huotari, J. and Helenius, A. (2011). Endosome maturation. *EMBO J.* **30**, 3481-3500.
Ibach, J., Radon, Y., Gelléri, M., Sonntag, M. H., Brunsveld, L., Bastiaens, P. I. and Verveer, P. J. (2015). Single particle tracking reveals that EGFR signaling activity is amplified in clathrin-coated pits. *PLoS ONE* **10**, e0143162.
Jiang, X., Huang, F., Marusyk, A. and Sorkin, A. (2003). Grb2 regulates internalization of EGF receptors through clathrin-coated pits. *Mol. Biol. Cell* **14**, 858-870.
John Peter, A. T., Lachmann, J., Rana, M., Bunge, M. Cabrera, M. and Ungermann, C. (2013). The BLOC-1 complex promotes endosomal maturation by recruiting the Rab5 GTPase-activating protein Msb3. *J. Cell Biol.* **201**, 97-111.
Johnson, D. E., Ostrowski, P., Jaumouillé, V. and Grinstein, S. (2016). The position of lysosomes within the cell determines their luminal pH. *J. Cell Biol.* **212**, 677-692.
Jongsma, M. L. M., Berlin, I., Wijdeven, R. H. M., Janssen, L., Janssen, G. M. C., Garstka, M. A., Janssen, H., Mensink, M., van Veelen, P. A., Spaapen, R. M. et al. (2016). An ER-associated pathway defines endosomal architecture for controlled cargo transport. *Cell* **166**, 152-166.
Jordens, I., Fernandez-Borja, M., Marsman, M., Dusseljee, S., Janssen, L., Calafat, J., Janssen, H., Wubbolts, R. and Neefjes, J. (2001). The Rab7 effector protein RILP controls lysosomal transport by inducing the recruitment of dynein-dynactin motors. *Curr. Biol.* **11**, 1680-1685.
Jordens, I., Marsman, M., Kuijl, C. and Neefjes, J. (2005). Rab proteins, connecting transport and vesicle fusion. *Traffic* **6**, 1070-1077.
Jorissen, R. N., Walker, F., Pouliot, N., Garrett, T. P. J., Ward, C. W. and Burgess, A. W. (2003). Epidermal growth factor receptor: mechanisms of activation and signalling. *Exp. Cell Res.* **284**, 31-53.
Kamio, T., Shigematsu, K., Sou, H., Kawai, K. and Tsuchiyama, H. (1990). Immunohistochemical expression of epidermal growth factor receptors in human adrenocortical carcinoma. *Hum. Pathol.* **21**, 277-282.
Kaplan, M., Narasimhan, S., de Heus, C., Mance, D., van Doorn, S., Houben, K., Popov-Čeleketić, D., Damman, R., Katrukha, E. A., Jain, P. et al. (2016). EGFR dynamics change during activation in native membranes as revealed by NMR. *Cell* **167**, 1241-1251.e1211.
Kourouniotis, G., Wang, Y., Pennock, S., Chen, X. and Wang, Z. (2016). Non-ligand-induced dimerization is sufficient to initiate the signalling and endocytosis of EGF receptor. *Int. J. Mol. Sci.* **17**, 1200.
Law, F., Seo, J. H., Wang, Z., DeLeon, J. L., Bolis, Y., Brown, A., Zong, W.-X., Du, G. and Rocheleau, C. E. (2017). The VPS-34 PI3 kinase negatively regulates RAB-5 during endosome maturation. *J. Cell Sci.* **130**, 2007-2017.
Lax, I., Burgess, W. H., Bellot, F., Ullrich, A., Schlessinger, J. and Givol, D. (1988). Localization of a major receptor-binding domain for epidermal growth factor by affinity labeling. *Mol. Cell. Biol.* **8**, 1831-1834.
Leevers, S. J., Vanhaesebroeck, B. and Waterfield, M. D. (1999). Signalling through phosphoinositide 3-kinases: the lipids take centre stage. *Curr. Opin. Cell Biol.* **11**, 219-225.
Lemberg, M. K. and Freeman, M. (2007). Cutting proteins within lipid bilayers: rhomboid structure and mechanism. *Mol. Cell* **28**, 930-940.
Leonard, D., Hayakawa, A., Lawe, D., Lambright, D., Bellve, K. D., Standley, C., Lifshitz, L. M., Fogarty, K. E. and Corvera, S. (2008). Sorting of EGF and transferrin at the plasma membrane and by cargo-specific signaling to EEA1-enriched endosomes. *J. Cell Sci.* **121**, 3445-3458.
Levkowitz, G., Waterman, H., Ettenberg, S. A., Katz, M., Tsygankov, A. Y., Alroy, I., Lavi, S., Iwai, K., Reiss, Y., Ciechanover, A. et al. (1999). Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1. *Mol. Cell* **4**, 1029-1040.
Li, X., Maretzky, T., Weskamp, G., Monette, S., Qing, X., Issuree, P. D. A., Crawford, H. C., McIlwain, D. R., Mak, T. W., Salmon, J. E. et al. (2015). iRhoms 1 and 2 are essential upstream regulators of ADAM17-dependent EGFR signaling. *Proc. Natl. Acad. Sci. USA* **112**, 6080-6085.
Li, H., You, L., Xie, J., Pan, H. and Han, W. (2017). The roles of subcellularly located EGFR in autophagy. *Cell. Signal.* **35**, 223-230.
Lin, X., Yang, T., Wang, S., Wang, Z., Yun, Y., Sun, L., Zhou, Y., Xu, X., Akazawa, C., Hong, W. et al. (2014). RILP interacts with HOPS complex via VPS41 subunit to regulate endocytic trafficking. *Sci. Rep.* **4**, 7282.
Luzio, J. P., Pryor, P. R. and Bright, N. A. (2007). Lysosomes: fusion and function. *Nat. Rev. Mol. Cell Biol.* **8**, 622-632.
Massague, J. and Pandiella, A. (1993). Membrane-anchored growth factors. *Annu. Rev. Biochem.* **62**, 515-541.
Mattera, R., Tsai, Y. C., Weissman, A. M. and Bonifacino, J. S. (2006). The Rab5 guanine nucleotide exchange factor Rabex-5 binds ubiquitin (Ub) and functions as a Ub ligase through an atypical Ub-interacting motif and a zinc finger domain. *J. Biol. Chem.* **281**, 6874-6883.
McEwan, D. G., Popovic, D., Gubas, A., Terawaki, S., Suzuki, H., Stadel, D., Coxon, F. P., Miranda de Stegmann, D., Bhogaraju, S., Maddi, K. et al. (2015). PLEKHM1 regulates autophagosome-lysosome fusion through HOPS complex and LC3/GABARAP proteins. *Mol. Cell.* **57**, 39-54.
Meister, M., Bänfer, S., Gärtner, U., Koskimies, J., Amaddii, M., Jacob, R. and Tikkanen, R. (2017). Regulation of cargo transfer between ESCRT-0 and ESCRT-I complexes by flotillin-1 during endosomal sorting of ubiquitinated cargo. *Oncogenesis* **6**, e344.
Moriki, T., Maruyama, H. and Maruyama, I. N. (2001). Activation of preformed EGF receptor dimers by ligand-induced rotation of the transmembrane domain. *J. Mol. Biol.* **311**, 1011-1026.
Navaroli, D. M., Bellve, K. D., Standley, C., Lifshitz, L. M., Cardia, L., Lambright, D., Leonard, D., Fogarty, K. E. and Corvera, S. (2012). Rabenosyn-5 defines the fate of the transferrin receptor following clathrin-mediated endocytosis. *Proc. Natl. Acad. Sci. USA* **109**, 471-480.
Neefjes, J., Jongsma, M. M. L. and Berlin, I. (2017). Stop or go? endosome positioning in the establishment of compartment architecture, dynamics, and function. *Trends Cell Biol.* **27**, 580-594.
Niendorf, S., Oksche, A., Kisser, A., Lohler, J., Prinz, M., Schorle, H., Feller, S., Lewitzky, M., Horak, I. and Knobeloch, K.-P. (2007). Essential role of ubiquitin-specific protease 8 for receptor tyrosine kinase stability and endocytic trafficking in vivo. *Mol. Cell. Biol.* **27**, 5029-5039.
Nordmann, M., Cabrera, M., Perz, A., Bröcker, C., Ostrowicz, C., Engelbrecht-Vandré, S. and Ungermann, C. (2010). The Mon1-Ccz1 complex is the GEF of the late endosomal Rab7 homolog Ypt7. *Curr. Biol.* **20**, 1654-1659.
Ogiso, H., Ishitani, R., Nureki, O., Fukai, S., Yamanaka, M., Kim, J.-H., Saito, K., Sakamoto, A., Inoue, M., Shirouzo, M. et al. (2002). Crystal structure of the complex of human epidermal growth factor and receptor extracellular domains. *Cell* **110**, 775-787.
Pankiv, S., Alemu, E. A., Brech, A., Bruun, J.-A., Lamark, T., Øvervatn, A., Bjørkøy, G. and Johansen, T. (2010). FYCO1 is a Rab7 effector that binds to LC3 and PI3P to mediate microtubule plus end-directed vesicle transport. *J. Cell Biol.* **188**, 253-269.
Penengo, L., Mapelli, M., Murachelli, A. G., Confalonieri, S., Magri, L., Musacchio, A., Di Fiore, P. P., Polo, S. and Schneider, T. R. (2006). Crystal structure of the ubiquitin binding domains of rabex-5 reveals two modes of interaction with ubiquitin. *Cell* **124**, 1183-1195.
Persaud, A., Alberts, P., Amsen, E. M., Xiong, X., Wasmuth, J., Saadon, Z., Fladd, C., Parkinson, J. and Rotin, R. (2009). Comparison of substrate specificity of the ubiquitin ligases Nedd4 and Nedd4-2 using proteome arrays. *Mol. Syst. Biol.* **5**, 333.
Pols, M. S., ten Brink, C., Gosavi, P., Oorschot, V. and Klumperman, J. (2013). The HOPS proteins hVps41 and hVps39 are required for homotypic and heterotypic late endosome fusion. *Traffic* **14**, 219-232.
Poteryaev, D., Datta, S., Ackema, K., Zerial, M. and Spang, A. (2010). Identification of the switch in early-to-late endosome transition. *Cell* **141**, 497-508.
Purba, E. R., Saita, E. and Maruyama, I. N. (2017). Activation of the EGF receptor by ligand binding and oncogenic mutations: the "rotation model". *Cells* **6**, 13.
Raiborg, C. and Stenmark, H. (2009). The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins. *Nature* **458**, 445-452.
Raiborg, C., Wenzel, E. M., Pedersen, N. M., Olsvik, H., Schink, K. O., Schultz, S. W., Vietri, M., Nisi, V., Bucci, C., Brech, A. et al. (2015). Repeated ER-endosome contacts promote endosome translocation and neurite outgrowth. *Nature* **520**, 234-238.

Raiborg, C., Wenzel, E. M., Pedersen, N. M. and Stenmark, H. (2016). ER-endosome contact sites in endosome positioning and protrusion outgrowth. *Biochem. Soc. Trans.* **44**, 441-446.
Rana, M., Lachmann, J. and Ungermann, C. (2015). Identification of a Rab GTPase-activating protein cascade that controls recycling of the Rab5 GTPase Vps21 from the vacuole. *Mol. Biol. Cell* **26**, 2535-2549.
Rappoport, J. Z. and Simon, S. M. (2009). Endocytic trafficking of activated EGFR is AP-2 dependent and occurs through preformed clathrin spots. *J. Cell Sci.* **122**, 1301-1305.
Rink, J., Ghigo, E., Kalaidzidis, Y. and Zerial, M. (2005). Rab conversion as a mechanism of progression from early to late endosomes. *Cell* **122**, 735-749.
Rocha, N., Kuijl, C., van der Kant, R., Janssen, L., Houben, D., Janssen, H., Zwart, W. and Neefjes, J. (2009). Cholesterol sensor ORP1L contacts the ER protein VAP to control Rab7-RILP-p150 Glued and late endosome positioning. *J. Cell Biol.* **185**, 1209-1225.
Robinson, M. S. (2015). Forty years of clathrin-coated vesicles. *Traffic* **16**, 1210-1238.
Row, P. E., Liu, H., Hayes, S., Welchman, R., Charalabous, P., Hofmann, K., Clague, M. J., Sanderson, C. M. and Urbé, S. (2007). The MIT domain of UBPY constitutes a CHMP binding and endosomal localization signal required for efficient epidermal growth factor receptor degradation. *J. Biol. Chem.* **282**, 30929-30937.
Rowland, A. A., Chitwood, P. J., Phillips, M. J. and Voeltz, G. K. (2014). ER contact sites define the position and timing of endosome fission. *Cell* **159**, 1027-1041.
Sabet, O., Stockert, R., Xouri, G., Brüggemann, Y., Stanoev, A. and Bastiaens, P. I. H. (2015). Ubiquitination switches EphA2 vesicular traffic from a continuous safeguard to a finite signalling mode. *Nat. Commun.* **6**, 8047.
Sahin, U., Weskamp, G., Kelly, K., Zhou, H.-M., Higashiyama, S., Peschon, J., Hartmann, D., Saftig, P. and Blobel, C. P. (2004). Distinct roles for ADAM10 and ADAM17 in ectodomain shedding of six EGFR ligands. *J. Cell Biol.* **164**, 769-779.
Sato, M., Sato, K., Fonarev, P., Huang, C.-J., Liou, W. and Grant, B. D. (2005). Caenorhabditis elegans RME-6 is a novel regulator of RAB-5 at the clathrin-coated pit. *Nat. Cell Biol.* **7**, 559-569.
Schink, K. O., Tan, K.-W. and Stenmark, H. (2016). Phosphoinositides in control of membrane dynamics. *Annu. Rev. Cell Dev. Biol.* **32**, 143-171.
Shan, Y., Eastwood, M. P., Zhang, X., Kim, E. T., Arkhipov, A., Dror, R. O., Jumper, J., Kuriyan, J. and Shaw, D. E. (2012). Oncogenic mutations counteract intrinsic disorder in the EGFR kinase and promote receptor dimerization. *Cell* **149**, 860-870.
Shinde, S. R. and Maddika, S. (2016). PTEN modulates EGFR late endocytic trafficking and degradation by dephosphorylating Rab7. *Nat. Commun.* **7**, 10689.
Sigismund, S., Woelk, T., Puri, C., Maspero, E., Tacchetti, C., Transidico, P., Di Fiore, P. P. and Polo, S. (2005). Clathrin-independent endocytosis of ubiquitinated cargos. *Proc. Natl. Acad. Sci. USA* **102**, 2760-2765.
Sigismund, S., Algisi, V., Nappo, G., Conte, A., Pascolutti, R., Cuomo, A., Bonaldi, T., Argenzio, E., Verhoef, L. G. G. C., Maspero, E. et al. 2013). Threshold-controlled ubiquitination of the EGFR directs receptor fate. *EMBO J.* **32**, 2140-2157.
Simonsen, A., Lippé, R., Christoforidis, S., Gaullier, J.-M., Brech, A., Callaghan, J., Toh, B.-M., Murphy, C., Zerial, M. and Stenmark, H. (1998). EEA1 links PI(3)K function to Rab5 regulation of endosome fusion. *Nature* **394**, 494-498.
Singh, A. B. and Harris, R. C. (2005). Autocrine, paracrine and juxtacrine signaling by EGFR ligands. *Cell. Signal.* **17**, 1183-1193.
Singh, B., Carpenter, G. and Coffey, R. J. (2016). EGF receptor ligands: recent advances. *F1000Research* **5**, 2270.
Sorkin, A., Mazzotti, M., Sorkina, T., Scotto, L. and Beguinot, L. (1996). Epidermal growth factor receptor interaction with clathrin adaptors is mediated by the Tyr974-containing internalization motif. *J. Biol. Chem.* **271**, 13377-13384.
Sousa, L. P., Lax, I., Shen, H., Ferguson, S. M., De Camilli, P. P. and Schlessinger, J. (2012). Suppression of EGFR endocytosis by dynamin depletion reveals that EGFR signaling occurs primarily at the plasma membrane. *Proc. Natl. Acad. Sci. USA* **109**, 4419-4424.
Stuible, M. and Tremblay, M. L. (2010). In control at the ER: PTP1B and the down-regulation of RTKs by dephosphorylation and endocytosis. *Trends Cell Biol.* **20**, 672-679.
Tan, X., Sun, Y., Thapa, N., Liao, Y., Hedman, A. C. and Anderson, R. A. (2015). LAPTM4B is a PtdIns(4,5)P2 effector that regulates EGFR signaling, lysosomal sorting, and degradation. *EMBO J.* **34**, 475-490.
Tomas, A., Futter, C. E. and Eden, E. R. (2014). EGF receptor trafficking: consequences for signaling and cancer. *Trends Cell Biol.* **24**, 26-34.
Traub, L. M. (2009). Tickets to ride: selecting cargo for clathrin-regulated internalization. *Nat. Rev. Mol. Cell Biol.* **10**, 583-596.
Ullrich, O., Reinsch, S., Urbe, S., Zerial, M. and Parton, R. G. (1996). Rab11 regulates recycling through the pericentriolar recycling endosome. *J. Cell Biol.* **135**, 913-924.
Ungermann, C., Price, A. and Wickner, W. (2000). A new role for a SNARE protein as a regulator of the Ypt7 Rab-dependent stage of docking. *Proc. Natl. Acad. Sci. USA* **97**, 8889-8891.
van der Kant, R., Fish, A., Janssen, L., Janssen, H., Krom, S., Ho, N., Brummelkamp, T., Carette, J., Rocha, N. and Neefjes, J. (2013). Late endosomal transport and tethering are coupled processes controlled by RILP and the cholesterol sensor ORP1L. *J. Cell Sci.* **126**, 3462-3474.
van der Kant, R., Jonker, C. T., Wijdeven, R. H., Bakker, J., Janssen, L., Klumperman, J. and Neefjes, J. (2015). Characterization of the mammalian CORVET and HOPS complexes and their modular restructuring for endosome specificity. *J. Biol. Chem.* **290**, 30280-30290.
Vieira, A. V., Lamaze, C. and Schmid, S. L. (1996). Control of EGF receptor signaling by clathrin-mediated endocytosis. *Science* **274**, 2086-2089.
Villasenor, R., Nonaka, H., Del Conte-Zerial, P., Kalaidzidis, Y. and Zerial, M. (2015). Regulation of EGFR signal transduction by analogue-to-digital conversion in endosomes. *Elife* **4**, e06156.
Wagner, M. J., Stacey, M. M., Liu, B. A. and Pawson, T. (2013). Molecular mechanisms of SH2- and PTB-domain-containing proteins in receptor tyrosine kinase signaling. *Cold Spring Harb. Perspect Biol.* **5**, a008987.
Watanabe, S. and Boucrot, E. (2017). Fast and ultrafast endocytosis. *Curr. Opin. Cell Biol.* **47**, 64-71.
Wijdeven, R. H., Jongsma, M. L. M., Neefjes, J. and Berlin, I. (2015). ER contact sites direct late endosome transport. *BioEssays* **37**, 1298-1302.
Wijdeven, R. H., Janssen, H., Nahidiazar, L., Janssen, L., Jalink, K., Berlin, I. and Neefjes, J. (2016). Cholesterol and ORP1L-mediated ER contact sites control autophagosome transport and fusion with the endocytic pathway. *Nat. Commun.* **7**, 11808.
Wiley, H. S. (2003). Trafficking of the ErbB receptors and its influence on signaling. *Exp. Cell Res.* **284**, 78-88.
Wu, P., Wee, P., Jiang, J., Chen, X. and Wang, Z. (2012). Differential regulation of transcription factors by location-specific EGF receptor signaling via a spatio-temporal interplay of ERK activation. *PLoS ONE* **7**, e41354.
Yasuda, S., Morishita, S., Fujita, A., Nanao, T., Wada, N., Waguri, S., Schiavo, G., Fukuda, M. and Nakamura, T. (2016). Mon1-Ccz1 activates Rab7 only on late endosomes and dissociates from the lysosome in mammalian cells. *J. Cell Sci.* **129**, 329-340.
Zeigerer, A., Gilleron, J., Bogorad, R. L., Marsico, G., Nonaka, H., Seifert, S., Epstein-Barash, H., Kuchimanchi, S., Peng, C. G., Ruda, V. M. et al. (2012). Rab5 is necessary for the biogenesis of the endolysosomal system in vivo. *Nature* **485**, 465-470.
Zerial, M. and McBride, H. (2001). Rab proteins as membrane organizers. *Mol. Cell Biol.* **2**, 107-119.
Zhang, Z., Zhang, T., Wang, S., Gong, Z., Tang, C., Chen, J. and Ding, J. (2014). Molecular mechanism for Rabex-5 GEF activation by Rabaptin-5. *Elife* **3**, e02687.
Zhu, Y., Serra, A., Guo, T., Park, J. E., Zhong, Q. and Sze, S. K. (2017). Application of nanosecond laser photolysis protein footprinting to study EGFR activation by EGF in cell. *J. Proteome. Res.* **16**, 2282-2293.