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New roles for endosomes: from vesicular carriers to multi-purpose platforms

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Abstract

The careful sorting and recycling of membranes and cargo and the intracellular delivery of proteins, toxins and viruses by endocytosis are well-established roles for the endocytic apparatus, which is present in all eukaryotic cells. Recently, it has become clear that endosomes have key roles in such diverse processes as cytokinesis, polarization and migration, in which their functions might be distinct from those classically associated with endosomes. We speculate that endosomes function as multifunctional platforms on which unique sets of molecular machines are assembled to suit different cellular roles.

The endocytic system comprises a series of compartments that have distinct roles in the sorting, processing and degradation of internalized cargo. These compartments, including early and recycling endosomes, multivesicular bodies, late endosomes and lysosomes, are connected with each other and the plasma membrane by mechanistically diverse and highly regulated pathways[1] (BOX 1). Endosomes efficiently recycle the large array of cargo that is taken up during endocytosis back to the cell surface or deliver it to other endocytic compartments[2]. Therefore, endosomes control many important physiological processes, including nutrient absorption, hormone-mediated signal transduction, immune surveillance and antigen presentation[1].

The role of endosomes in sorting and trafficking is facilitated by their highly dynamic composition and morphology[3]. Localized production of specific lipids (such as phosphatidylinositol-3-phosphate (PtdIns3P) by class II or class III phosphoinositide 3-kinases (PI3Ks)) on endosomes causes the recruitment of molecules (such as early endosome antigen 1 (EEA1), and RAB5 (BOX 1) and its effectors) to subdomains of endosomal membranes[4,5]. This facilitates the formation of many different membrane

DATABASES
UniProtKB: http://www.uniprot.org
ALIX | ARF6 | CDC-42 | CHMP1B | EEA1 | ENDO180 | PAR-3 | PAR-6 | PKC-3 | PTEN | RAB4 | RAB5 | RAB8 | RAB11 | RAB35 | ROCK1 | SARA | SDF1 | TIAM1 | TSG101 | WNT5A

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domains, allowing coordinated membrane fusion, tubulation and invagination of endosomal membranes during uptake and sorting of extracellular components[6].

Endosomes are now also recognized to have important roles in cellular signalling. Endosomes function to terminate signalling processes (for example, through the internalization of receptors) and function in signal propagation by facilitating the recruitment and integration of signalling cascades on the surface of endocytic vesicles[7–9] (BOX 2).

Here, we propose the idea that endosomes, in addition to their well-characterized role as entry portals, sorting stations and signalling foci, also function as multifunctional platforms on which unique sets of molecular machines are assembled. In this way, endosomes participate in a range of processes, including cell signalling, cytokinesis, polarity and migration.

Endosomes in cytokinesis

Cytokinesis is the final stage of mitosis, during which the two daughter cells physically separate. After replication of the genetic material, a furrow forms between the two cells and constricts using an actomyosin-based contractile ring, leaving the daughter cells attached by a thin bridge. The resolution of this bridge, by a process called abscission, separates the two cells[10,11]. Recent studies have implicated both secretory and endosomal trafficking processes in these events[10]. Although it was initially assumed that this trafficking is associated with the increase in plasma membrane area in dividing cells, subsequent analyses have shown that membrane trafficking also has a central role in the abscission process[10–12]. So, what role might endosomes have in this event?

An assembly point for the abscission machinery?

One possibility is that endosomes serve as focal points for the assembly of the abscission machinery. In late telophase, both secretory and endocytic vesicles move rapidly into the midbody and accumulate in the intercellular bridge immediately adjacent to the midbody ring[13]. Vesicles from both daughter cells traffic from the Golgi into the furrow[14]. Moreover, these vesicles accumulate in the midbody, thereby generating a tightly packed pool of vesicles that can dock and fuse with the plasma membrane[14]. Real-time analysis of these events reveals an even more striking observation — the fusion of these vesicles, both with each other and with the surrounding plasma membrane (a process known as compound fusion), precedes abscission[13].

Endocytosis also has a central role in abscission itself, as RAB11-positive endosomes have been shown to traffic from centrosomes into the furrow from both daughter cells[15]. RAB35 has also been implicated in abscission, as it might control the trafficking of septins (a family of evolutionary conserved guanine nucleotide-binding proteins that can form filaments) and lipid remodelling activity into the area of the growing furrow and midbody, perhaps regulating levels of phosphoinositides[16]. Phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) accumulates at the cleavage furrow[17–19], whereas phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3) is enriched at the poles of the dividing cells[20]. The two enzymes that control the distribution of PtdIns(3,4,5)P_3 — PTEN (phosphatase and tensin homologue) and class I PI3K — are enriched in the furrow and the poles of the dividing cells[20], respectively, and cells that lack these enzymes fail to complete cytokinesis[20]. Such data might imply that endosomal vesicles could provide a unique platform for the assembly of the abscission machinery, perhaps involving the assembly of complex lipid remodelling activities on the endosomal surface. This represents a novel function of endosomes.

BRUCE and midbody endosomes

This view is supported by recent studies of a large multi functional protein called BRUCE[21]. BRUCE is thought to act as a platform that regulates the delivery of endosomal and secretory vesicles in the midbody, and its function is essential for abscission. In addition to regulating vesicle docking and accumulation in the midbody, it has been speculated that BRUCE might act as a diffusion barrier that effectively 'separates' the two daughter cells before abscission[21]. BRUCE might then serve to anchor endosomal vesicles in the midbody at high density, thereby facilitating the assembly of the abscission machine on their surface. BRUCE also contains ubiquitin-conjugating activity, and ubiquitin relocalizes from midbody microtubules to the midbody ring in telophase[21]. Ubiquitin has an important role in membrane trafficking (BOX 1), and thus the ubiquitin-conjugating activity of BRUCE might in turn regulate the abscission machine assembled on these anchored endosomes.

Role of the ESCRT pathway

ESCRT (endosomal sorting complex required for transport) I, II and III are multiprotein complexes that recognize ubiquitylated cargo in the endosomal system and direct this cargo into multivesicular bodies (BOX 1). Further evidence that endosomal vesicles provide a platform for assembly of the abscission machinery during cytokinesis comes from recent data that implicate the ESCRT pathway in cytokinesis[22]. TSG101 (a subunit of the ESCRTI protein complex) and the ESCRT-interacting protein ALIX (apoptosis-linked gene 2-interacting protein X) are recruited by interaction with CEP55 to the midbody, where they interact with several other key cytokinesis proteins, notably Rho-associated kinase 1 (ROCK1) and the IQGAP proteins[23].

In addition to ESCRTI and ALIX, ESCRTIII has emerged as a potential player in cytokinesis. ESCRTIII proteins form circular arrays or tubes[24] that participate in the membrane abscission step of cytokinesis[25,26]. This could occur by regulating microtubule severing, as recent work has shown that the microtubule-severing protein spastin binds to the ESCRTIII subunit charged multivesicular body protein 1B (CHMP1B)[27]. CHMP1B recruits spastin to the midbody, which suggests that, once localized, spastin could facilitate cytokinesis by severing midbody microtubules[27].

Not just plasma membrane expansion

So, what can we discern about the roles of endosomes in cytokinesis from these studies? Although the initial view that endosomes contribute to plasma membrane expansion is likely to be valid[28], we suggest that further roles for endosomes should be posited (FIG. 1). Endosomal trafficking into the midbody is probably needed to deliver key cargo that is required for lipid remodelling and plasma membrane dynamics during furrowing (clearly, there needs to be careful orchestration of membrane deformation and actomyosin ring contraction)[29]. We suggest that the assembly of a pre-abscission 'platform' of densely packed endosomal and secretory vesicles in the midbody acts as a diffusion barrier before abscission and facilitates the formation of the abscission machinery, perhaps involving ubiquitylation or de-ubiquitylation events. The above data support the idea of an endosomally localized complex on densely packed vesicles in the midbody that is central to abscission. Further work in this area is needed to understand how endocytic vesicles specifically target to the midbody (including the roles of the cytoskeleton and molecular motors), the identity of the abscission machine components and how these components function in directing abscission.

Endosomes and polarity

A wide range of cells polarize in response to specific signals by reorganizing their cellular components. This is important in, for example, the establishment of apical and basolateral surfaces in epithelial cells, and also in the asymmetric distribution of developmental determinants during cell division. Membrane trafficking is essential for the generation and maintenance of polarity[30,31], and this link has been reinforced by a recent study that shows that clathrin, a key protein involved in endocytosis (BOX 1), also regulates basolateral polarity in mammalian cells[32]. Knockdown of clathrin using small interfering RNA was found to slow the exit of basolateral proteins from the Golgi complex, resulting in their missorting into apically targeted transport vesicles[32]. But do endosomes (and endocytosis) have other roles in polarity determination? Recent exciting studies suggest that they do.

Endocytic trafficking and PAR proteins

A central role for the partitioning defective (PAR) proteins in polarity determination has been established in worms, flies and humans[33]. Subsets of PAR proteins become asymmetrically localized, and understanding how this is achieved is a central goal of polarity research. New studies have surprisingly implicated endosomes as potential players in this process. A genome-wide screen for endocytic regulators in *Caenorhabditis elegans* uncovered 168 candidate genes. Strikingly, a subset of these were found to encode proteins that are already known to be central mediators of polarity, specifically PAR-3, PAR-6, protein kinase C-like 3 (PKC-3) and CDC-42 (REF. 34). Endosomal trafficking was compromised when these genes were disrupted both in worms and HeLa cells[34]. This elegant study provides a clear link between polarity proteins and endocytic trafficking and epithelial polarity.

So, how might such observations be rationalized? As PAR proteins are linked to the cortical actomyosin network in *C. elegans*, it was suggested that PAR proteins might regulate endocytic trafficking by controlling actin dynamics[34]. Such a model is certainly plausible, but there are additional possibilities.

Such studies are suggestive of a requirement to assemble populations of endosomes for defined cellular functions. It is possible that this is especially true for polarity determination, and that the link between endocytic trafficking, the PAR proteins and the actomyosin network reflects a need to assemble populations of endosomes that localize polarity signals with the machinery required to control polarity. Therefore, by extension of the ideas outlined above for cytokinesis, endocytic trafficking might provide a platform on which key molecular machines can be assembled. In a striking parallel with the data on cytokinesis outlined above, the ESCRT machinery has been shown to be a crucial determinant of polarity[35–37]. Regardless of the precise mechanism, endocytic trafficking and polarity seem to be inextricably linked.

Consistent with this view, a recent report has identified a novel mechanism that involves the intercellular transfer of specialized membrane domains to signalling endosomes for the targeted regulation of signalling and remodelling events in the haematopoietic niche microenvironment[38]. Haematopoietic stem and progenitor cells in this study were found to make prolonged contact with the osteoblast surface through a specialized membrane domain that is enriched in prominin 1, CD63 and rhodamine-phosphatidylethanolamine. At the contact site, portions of the specialized domain that contain these molecules were taken up by the osteoblast and internalized into long-lived, SARA (SMAD anchor for receptor activation; also known as ZFYVE9)-positive signalling endosomes[38] (BOX 2). This downregulates osteoblast SMAD signalling and increases the production of stromal-derived factor 1 (SDF1; also known as CXCL12), a chemokine that is responsible for the homing of

haematopoietic stem and progenitor cells to bone marrow[38]. These findings identify a novel mechanism that involves the intercellular transfer of specialized membrane domains to signalling endosomes for the targeted regulation of signalling and remodelling events within the osteoblastic niche microenvironment.

Endosomes in cell migration

Endocytic trafficking is now established as a crucial mechanism to control cell signalling in growth and development, in part by concentrating signalling molecules in defined intracellular regions (BOX 2). Recent work has suggested that endosomes might fulfil additional functional roles in migration that are similar to those described above for endosomes in cytokinesis or polarity.

Endosomes and rear-end migration

The assembly and disassembly of focal adhesions and adherens junctions are crucial facets of cell motility and tumour invasion[39]. These are controlled through the Rho-dependent activation of Rho kinase, which in turn phosphorylates myosin light chain 2 and thereby generates the contractile force that is necessary to disassemble cell–cell junctions and cell–matrix adhesions at the rear of the migrating cell[39]. Recent studies of this system suggest that populations of endosomes that contain the pro-migratory receptor ENDO180 (also known as MRC2) become localized to sites of adhesion at the rear of cells[40]. This redistribution is not simply a result of changing the rates of ENDO180 recycling, but rather arises from a redirection of endosomal trafficking into this specific location. ENDO180 generates the Rho kinase-derived contractile signals that are needed for adhesion disassembly; hence, migrating cells can spatially localize the required cellular machinery for focal adhesion turnover using endosomes as a platform[40] (FIG. 2).

An interesting extension of such studies has arisen from efforts to understand Wnt signalling[41]. Wnt signalling controls both cell polarity and directional cell movement, but the mechanisms by which Wnt pathways exert such control are incompletely understood. New studies have identified a so-called Wnt-mediated receptor–actin–myosin polarity (W-RAMP) structure, which accumulates asymmetrically at the cell periphery in response to WNT5A[41]. This structure seems to trigger membrane contractility and nuclear movement in the direction of membrane retraction. W-RAMP formation involves the recruitment of actin, myosin IIB and the Wnt ligand receptor frizzled 3, is dependent on RAB4 and RAB8, requires endosome trafficking, and is associated with multivesicular bodies[41]. Membrane-organized W-RAMPs could then be a further example of an endosome-localized membrane platform for a specific cellular function.

Endosomes lead from the front

Another important role for endosomes in cell migration has recently been identified — this time at the other end of the cell. The formation of lamellipodia, waves or circular dorsal ruffles is important in cell migration, is controlled by small GTPases such as Rac, and is known to involve endocytic proteins, such as dynamin and the early endosomal protein RAB5 (REF. 42). Recent studies have provided new mechanistic insights into the roles of endosomes in these events[43], revealing that RAB5-dependent endocytosis is required for activation of Rac induced by motogenic stimuli, and that this activation occurs on endosomes that also contain the Rac guanine nucleotide-exchange factor TIAM1 (FIG. 2).

Rac activation needs to be controlled spatially to drive the formation of specific protrusions (such as circular dorsal ruffles). Cells seem to achieve this by using endosomes as a specific platform to spatially localize the Rac activation machinery. Although at first this might seem to reflect solely a signalling role for endosomes, the story is more complex. Rac-containing

vesicles are transported from endosomes to cell surface ruffles in an ARF6-dependent step[43] (ARF6 is a monomeric GTPase that is implicated in membrane trafficking; BOX 1). Hence, it would seem reasonable to propose that, in this particular case, distinct endosomes act to assemble machines to serve the complex interlinked functions of activating Rac, selecting integrin cargoes and targeting vesicles to ruffles. Such studies further support the notion that endosomes, localized to distinct cellular environments, can be used to assemble machines for specific functions.

How endosomes become platforms

It has long been appreciated that the negative surface charge of the plasma membrane, which arises from the presence of phosphoserine (10–20% of all surface lipid) and phosphoinositides, has a crucial role in the targeting of proteins that contain polycationic motifs[44]. Exciting studies have shown that receptor activation can alter the inner surface potential during phagocytosis, resulting in the rapid release of molecules, such as KRAS, RAC1 and Src from the membrane[45]. Modulation of surface charge can also redirect proteins that are normally resident on plasma membranes to endosomal compartments[46].

We speculate that similar alterations in the surface charge (possibly arising from alterations in lipid composition) on endosomes that are localized in distinct cellular regions could facilitate distinct ‘endosome functionality’, serving to recruit, for example, the abscission machinery on to accumulated vesicles in the midbody, or polarity signals on to vesicles tethered in one region of a cell. Ubiquitin might also have a role in this regard, perhaps by facilitating the interaction of other proteins with ubiquitylated endosomal cargo or by modifying the behaviour of endosome-localized proteins. A challenge for the years ahead will be to define the molecular basis of such interactions.

Studies such as those discussed above offer the hypothesis that, by using endosomal membranes as a platform, cells could assemble specific ‘machines’ in space and time. This should lead to a change in the view of an endosome from a ‘simple’ vesicular carrier to a more complex and dynamic ‘organelle of many coats’.

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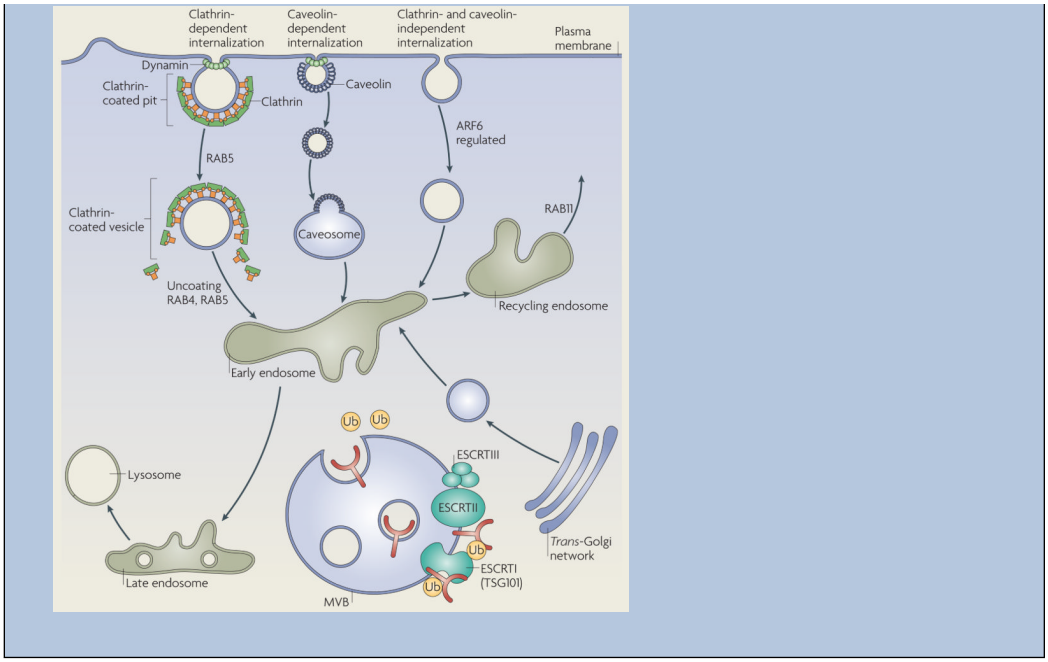
Box 1 | Pathways of endocytosis

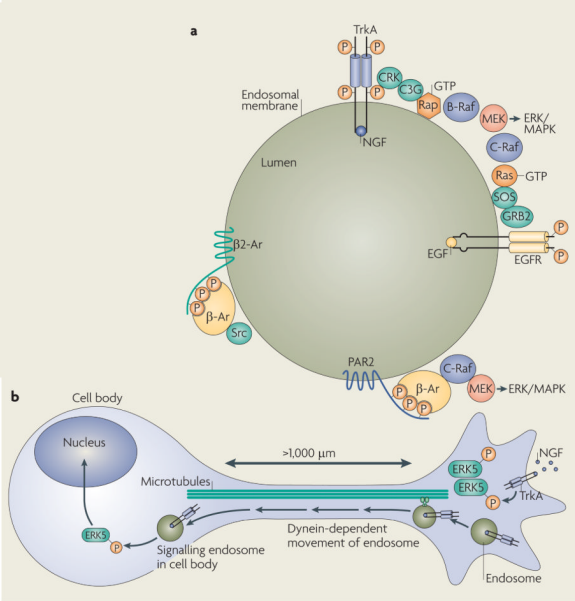
Receptors, ligands, viruses and other endocytosed molecules are internalized using several routes (see the figure). In the classic pathway of clathrin-dependent endocytosis, receptors and their bound ligands are sorted into specialized domains of the plasma membrane through interactions with adaptor protein complexes. These areas invaginate and are coated with the scaffold protein clathrin. The interaction of the adaptors with the GTPase dynamin results in the formation of a ring of dynamin around the neck of the forming clathrin-coated pit, leading to scission and the formation of clathrin-coated vesicles. These vesicles then uncoat and fuse with early endosomes[47]. This pathway is used by recycling receptors, such as transferrin and low-density lipoprotein receptors. Caveolin-dependent endocytosis is important for the uptake of toxins, viruses, bacteria and circulating proteins[48]. This does not involve clathrin, but requires the scaffold protein caveolin. Dynamin is also important for the scission of caveolin-containing vesicles, known as caveosomes, which can fuse with early endosomes. A third, distinct mechanism of internalization does not use clathrin, caveolin or dynamin[48] but requires the action of the GTPase ARF6. Cargo that is internalized by this pathway includes integrins, major histocompatibility complex molecules and glycosyl phosphatidylinositol-anchored proteins. Once in early endosomes, receptors and other cargo can then be sorted either into late endosomes and lysosomes, or recycled back to the cell surface. Monoubiquitylated membrane proteins destined for downregulation are recognized by a series of receptors containing monoubiquitin-binding domains. These receptors target monoubiquitylated membrane proteins through a series of trafficking steps that ultimately deliver them to be destroyed in the lysosome. This requires an invagination of the endosome membrane that forms multivesicular bodies (MVBs). Ubiquitin (Ub) functions as a key signal for sorting into MVBs. Endosomal membrane proteins that are destined for degradation in the lysosome are actively sorted into the intraluminal vesicles of a MVB, whereas proteins destined for the limiting membrane of the lysosome are retained in the limiting membrane of the MVB. ESCRT (endosomal sorting complex required for transport) complexes have a crucial role in this process[49]. TSG101 is a subunit of the ESCRTI protein complex.

Rab proteins are members of the Ras superfamily of monomeric GTPases. Different Rabs regulate many facets of membrane trafficking, including vesicle formation and movement and membrane fusion, and are localized to distinct endosomal compartments.



Box 2 | Endosomes as signalling foci

Endocytosis has long been recognized as a mechanism for terminating signalling by degrading activated receptors. It is now well established that the output from any given signalling pathway is dependent on where the signal is localized. Endosomes are now established as key foci for the assembly of signalling complexes, suggesting that nature has taken advantage of the compartmentalization of the endocytic pathway to localize signals in space. Examples shown (see the figure, part **a**) include the recruitment of CRK–C3G–Rap or GRB2–SOS and the activation of the MEK–ERK cascade from NGF receptors (indicated as TrkA) and EGFR, respectively, through different Raf kinase isoforms (B-Raf and C-Raf) (EGFR stands for epidermal growth factor receptor, ERK for extracellular signal-regulated kinase, MEK for mitogen-activated protein kinase/ERK kinase and NGF for nerve growth factor). Also shown are two examples of G-protein-coupled receptor signalling complexes localized to endosomes. Internalized protease-activated receptor 2 (PAR2; also known as F2RL1) uses β-arrestins (β-Ars) to activate the MEK–ERK cascade. Internalized β-adrenoceptors (β2-Ars) recruit β-arrestins, which in turn interact with non-receptor Tyr kinases, such as Src (for recent reviews, see REFS 7,8,9,50). A striking example of the importance of endosome-localized signalling is provided by studies of TrkA signalling in neurons. The application of NGF (the TrkA ligand) to distal axons results in activation of ERK5 (a member of the MAPK family) in the cell body by a mechanism that is thought to involve the trafficking of internalized receptors on endosomes into the cell body[9] (see the figure, part **b**).



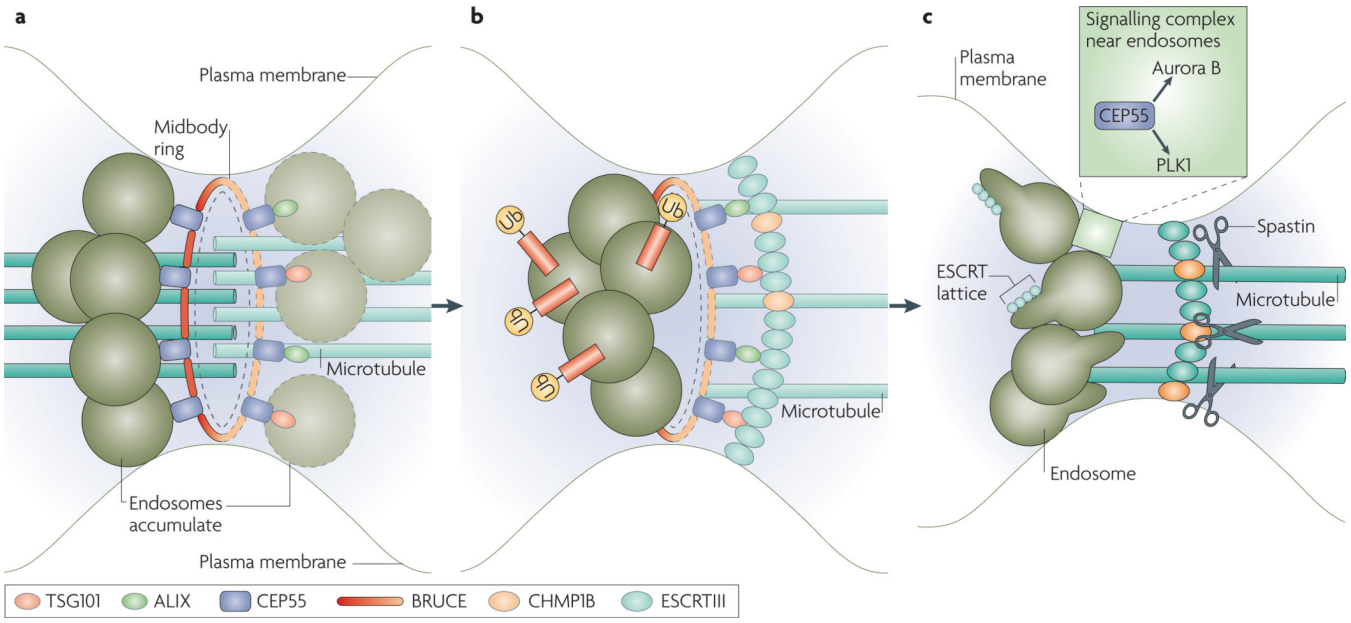


Figure 1. Endosome platforms in cytokinesis

For clarity, endosomal trafficking to the midbody and the assembly of the CEP55–ALIX–ESCRT complex (where ALIX is apoptosis-linked gene 2-interacting protein X and ESCRT is endosomal sorting complex required for transport) are drawn on opposite sides of the midbody. In reality, such mechanisms probably exist on both 'sides' of the midbody ring. **a** | During late telophase, RAB11- and RAB35-positive endosomes accumulate in the midbody by interacting with the vesicle-tethering exocyst complex, which is localized to the midbody ring by centriolin (not shown) and perhaps by interacting with BRUCE (see the main text). BRUCE is delivered to the midbody on membrane vesicles, on which it can also function as a diffusion barrier, preventing vesicle movement through the furrow. Collectively, these interactions anchor vesicles in the midbody. **b** | BRUCE is a ubiquitin ligase through which endosomal cargo might become ubiquitylated. CEP55 recruits ALIX and TSG101, which are components of ESCRTI, into the midbody. ESCRTII and ESCRTIII might also become localized here, perhaps through interactions with ALIX or TSG101, or with ubiquitylated endosomal cargo. These events might occur at the same time as vesicle docking (part **a**). **c** | ESCRT proteins form a lattice on endosomal membranes and induce membrane deformation. ESCRT complexes might also facilitate sorting away from midbody-localized endosomes, perhaps removing a 'fusion brake'. The microtubule-severing protein spastin binds to the ESCRTIII subunit CHMP1B (charged multivesicular body protein 1B). These events (lattice formation and recruitment of spastin) might take place on endosomal surfaces, providing a degree of spatial resolution. In response to a signal, perhaps from an adjacent signalling complex, endosomes (and secretory vesicles) undergo compound fusion. CEP55 in the midbody ring might also function to recruit signalling proteins, such as Aurora B and Polo-like kinase 1 (PLK1), into this region. If spatially and temporally coupled to spastin cleavage of microtubules, this might constitute the basic machinery of abscission.

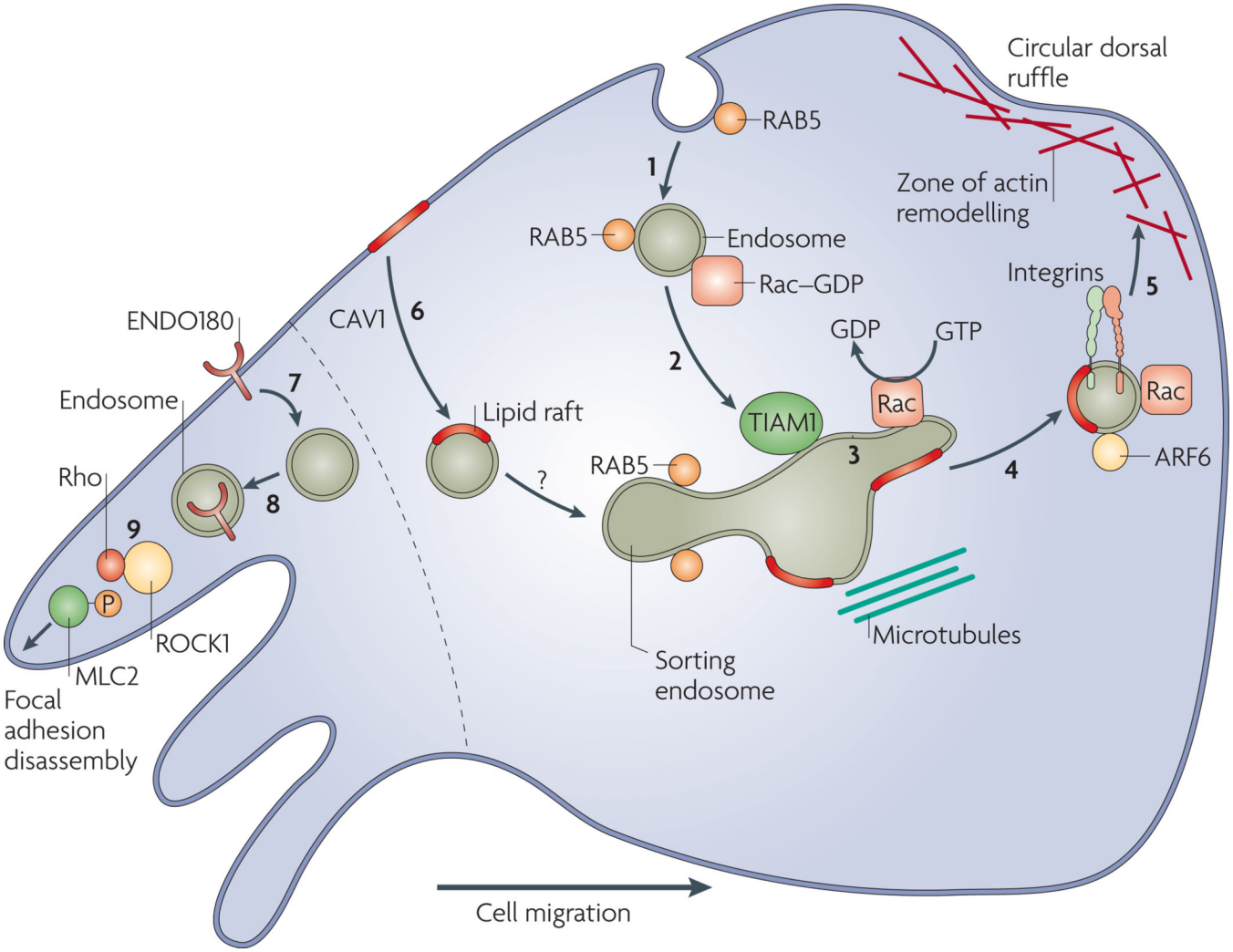


Figure 2. Endosome platforms in cell migration

The schematic shows a cell that is directing traffic towards circular dorsal ruffles (right) and at the same time disassembles focal adhesions at the rear of the cell (left). RAB5- and dynamin-dependent internalization of cell surface cargo (including Rac) into endosomes is shown (steps 1,2). The endosomes, which are characterized by RAB5, traffic Rac into an endosomal subcompartment that contains the Rac guanine nucleotide-exchange factor TiAM1, resulting in GTP loading, and activation, of Rac (step 3). Vesicles bud off of this compartment and contain activated RAC1 for forward-directed trafficking to the zone of ruffling (step 4). These vesicles might also contain integrin cargo and possibly lipid raft domains. The GTPase ARF6 has been implicated in the trafficking of both rafts and integrins, and hence it is possible that the intracellular endosomal compartment is a focal point for the assembly of an actin-remodelling ‘machine’ that traffics in an ARF6-dependent manner, possibly along microtubules towards the ruffles (step 5). Rafts are known to recycle through caveolae, which contain the protein caveolin 1 (CAV1; step 6). This recycling involves ARF6, suggesting a possible link between these two processes. Focal adhesion disassembly at the rear of the cell might also involve endosome platforms. The pro-migratory receptor ENDO180 (also known as MRC2) traffics between the cell surface and endosomes (step 7). On an appropriate cue, ENDO180 traffic is redirected into endosomes at the rear of the cell near focal adhesions, and serves as a platform for the assembly of Rho–

ROCK1–MLC2-derived contractile signals (where MLC2 is myosin light chain 2 and ROCK1 is Rho-associated kinase 1) directly at sites of focal adhesions (steps 8,9).