Endocytosis in the context-dependent regulation of individual and collective cell properties

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Abstract

Endocytosis allows cells to transport particles and molecules across the plasma membrane. In addition, it is involved in the termination of signalling through receptor downmodulation and degradation. This "traditional" outlook has been substantially modified, in recent years, by discoveries that endocytosis and subsequent trafficking routes have a profound impact on the positive regulation and propagation of signals, being key for spatio-temporal regulation of signal transmission in cells. Accordingly, endocytosis and membrane traffic regulate virtually every aspect of cell physiology, and are frequently subverted in pathological conditions. Two key aspects of endocytic control over signalling are coming into focus: context-dependency and long-range effects. First, endocytic-regulated outputs are not stereotyped, but heavily dependent on cell-specific regulation of endocytic networks. Second, endocytic regulation has impact not only on individual cells, but also on the behaviour of cellular collectives. Herein, we will discuss recent advancements in these areas, highlighting how endocytic trafficking impacts complex cell properties, including cell polarity and collective cell migration, and the relevance of these mechanisms to disease, in particular cancer.

[H1] Introduction

Endocytosis is used by cells to internalize various types of molecules, including nutrients, and fluids, which could not otherwise pass through the plasma membrane[1,2]. While this has probably represented the initial driving force behind its emergence in evolution, the system has been exploited to actively regulate various forms of communication within the cell and between the cell and its environment. Signalling receptors, for instance, are internalized upon engagement by cognate ligands and frequently targeted for degradation in the lysosome, resulting in long-term signalling attenuation[3,4]. In addition, regardless of their interaction with extracellular moieties, many surface-resident molecules (mostly, but not exclusively, proteins) are

internalized and either degraded or recycled back to the cell surface, thereby providing a mechanism through which the cell controls and adjusts its repertoire of plasma membrane-resident molecules for various functional purposes. Yet, these are only particular facets of endocytosis, whose impact on cellular homeostasis appears much wider (see Supplementary Table 1).

A modern view of endocytic trafficking is that of a "vast program, deeply engrained in the cellular masterplan and inextricably intertwined with signaling, which constitutes the major communication infrastructure in the cell"[1]. At the individual cell level, for instance, endosomes represent major signalling stations. This is embodied in the concept of the "signalling endosome": a platform capable of sustaining signalling by numerous mechanisms, including assembly of endosome-specific signalling complexes, crosstalk, regulation of signal persistence in intracellular compartments, and signal computing and resolution in time and space[5]. Endocytosis also controls the execution of polarized cell functions through the redistribution of surface molecules towards sites of polarized activities. In this case, fast and site-directed redistribution of membrane proteins is not achieved by planar diffusion on the plasma membrane, but rather by cycles of endocytosis and directed recycling[6-9].

Although these activities are largely pertinent to the workings of individual cells, it is becoming increasingly clear that also cellular collectives are controlled by endocytosis. This is remarkable, as it entails that endocytic events occurring on the level of a single cell must be synchronized, frequently spanning a distance of hundreds of cells, to contribute to a coordinated behaviour[10-13].

Mechanistically, endocytosis has long been considered a rather stereotyped process, irrespective of the cell type and (to some extent) of the transported cargo. This vision started to change with the realization that clathrin-coated pits, responsible for clathrin-mediated endocytosis (CME), have varying compositions and contain a plethora of non-obligatory components that confer specificity to the uptake process. In addition, a wealth of non-clathrin endocytosis (NCE) routes exist, which also exhibit context specificity, being present only in certain cell types[2,15,16]. This heterogeneity in endocytic mechanisms is reflected in differences in effector functions in cells, both at the individual and the collective cellular level, which increasingly appear to depend on the varying "endocytic landscapes" of different cell types. With this expression, we mean the varying cellular composition in endocytic and trafficking proteins that can determine the presence or absence of a certain endocytic route and/or confer a different cargo specificity of the same endocytic route in different cells. In turn, this may determine diverse biological outcomes in response to the same signalling input, as a function of the cellular endocytic context.

Given the pervasive nature of endocytosis and its emerging roles in the control of virtually every cellular phenotype (Supplementary Table 1), it is not surprising that its subversion is relevant to human pathologies[1,17,18]. As one example, in cancer, the context-dependency of endocytic control over signalling might have a major role in the migratory/invasive phenotype. Indeed, endocytic pathways seem to be preferentially involved in the acquisition of pro-metastatic traits *vs*. other tumor properties[6,18] (see Supplementary Tables 2 and 3).

In this Review, we discuss the pleiotropic roles of endocytosis in cell regulation and the importance of these mechanisms in physiology and pathology, especially cancer. Many of these facets of endocytosis have been excellently reviewed elsewhere[5-7,10-13].

Herein, we will concentrate on an emerging trait: that of context-dependency of endocytic regulation, with respect to different cell types and different stimuli they are exposed to. We will also highlight the emerging view that endocytic trafficking, although occurring at the single-cell level, has an impact on tissue-wide properties and behaviours, such as tissue polarity and collective cell migration.

[H1] Endocytic regulation in context

In this section, we will use paradigmatic examples to illustrate how the greater context – encompassing type of cargo as well as cell type and expression of proteins comprising endocytic machinery – in which endocytosis operates influences its impact on cell function in physiology and cancer. We will concentrate predominantly on the regulation of signalling by receptor tyrosine kinases (RTKs) and G protein-coupled receptors (GPCRs). For discussions on the impact of endocytic routes on intracellular communication mediated by membrane contact sites[19-21], on endocytosis in neuronal communication and synaptic function[22-24], and on the trafficking of adhesion molecules, such as integrins and cadherins[25-27], we refer the reader to several exhaustive reviews.

[H2] Differential regulation of signalling by distinct clathrin-coated structures.

Different types of clathrin-coated structures exist at the surface of mammalian cells. These include dynamic curved clathrin-coated pits that are heterogeneous in dimension, dynamics and composition and are responsible for constitutive and ligand-induced CME of different cargoes (Figure 1) as well as long-lived large flat clathrin lattices, also known as coated plaques[28-33], which act as signalling and adhesion platforms[34], in addition to other functions (Box 1).

CME is characterized by a core of molecular components, prominently including: clathrin; its main adaptor, adaptor protein 2 (AP2), which bridges cargoes to clathrin[35,36]; and the GTPase, dynamin, responsible for endocytic vesicle fission[37,38] (see example in Figure 1a). Depending on the cellular context and the type of cargo, other adaptors — such as DAB2, ARH, epsins, EPS15 and EPS15L1 as well as arrestins —can link cargoes to the clathrin machinery, producing clathrin-coated pits with varying characteristics[14,36,39] (see example in Figure 1b). In addition, AP2 levels are regulated in a cell context-dependent manner, impinging on CME dynamics[40] and on fate(s) and function(s) of signalling receptors (see below). Thus, the view that clathrin-coated pits represent a uniform population is now outdated; it is now evident that distinct clathrin-coated pits exist, with differential roles in the control of receptor fate and signalling[28].

This is exemplified by the regulated endocytosis of GPCRs, which occurs preferentially through subsets of distinct clathrin-coated pits characterized by: the engagement of specific adaptors (such as β-arrestins)[41]; specific regulation, (such as receptor ubiquitylation)[41]; increased surface residence time/slow internalization rates (achieved by receptor–actin cytoskeleton interactions) (Figure 1c)[42-44]. GPCR recruitment to specific subsets of clathrin-coated pits and cargo (ligand-bound GPCRs)-dependent control of clathrin-coated pit dynamics and composition, are thought to reduce competition with other CME cargoes, allowing the generation of clathrin-coated pits with specialized functions[42]. Interestingly, β-arrestin-mediated CME varies

depending on the type of GPCR that is activated, as exemplified by the β-subfamily of adrenergic receptors (AR). At variance with the other family members, the β1-AR is endocytosis-incompetent, meaning it is not internalized, yet, is still able to induce β-arrestin accumulation and signalling in clathrin-coated pits[45-47]. Although the molecular mechanisms responsible for this accumulation and activation of β-arrestin are not completely elucidated, computational simulations suggest that the active conformation of β-arrestin is stabilized by binding to the receptor tail and persists even after detachment from the receptor; this conformational change renders β-arrestin competent for binding to clathrin and AP2[47].

Similar to GPCRs, the epidermal growth factor receptor (EGFR) is also internalized through distinct classes of clathrin-coated pits, distinguished by the presence or absence of AP2 (Figure 1d), which differently regulate EGFR recycling/degradation and thereby signalling, in a context-specific fashion (*e.g.*, fibroblasts *vs*. epithelial cells), depending on the type of internalizing clathrin-coated pit (AP2-proficient or AP2-less)[48-51]. Specialized and context-specific functions of clathrin-coated pits also appear to be conferred by the different members of the epsin family of endocytic adaptor proteins [52-54]. Epsin1 (EPN1) and EPN2 are ubiquitously expressed and are implicated in the CME of several plasma membrane receptors, such as the aforementioned EGFR, as well as vascular endothelial growth factor receptors (VEGFR), NOTCH and WNT receptors[48,55-60]. By contrast, EPN3 shows a more restricted pattern of expression, suggesting cell-specific functions[52,61]. Indeed, EPN3 has a selective role in the regulation of E-cadherin (CDH1) endocytosis and turnover and, when amplified/overexpressed, contributes to breast cancer development through the induction of a partial epithelial- mesenchymal transition (EMT) state (see also subsection Endocytosis and EMT below)[53].

Different dynamin isoforms also mark distinct types of clathrin-coated pits. Dynamin1 (DYN1) has traditionally been viewed as neuronal-specific and involved in synaptic vesicle recycling, where its activity is regulated through phosphorylation–dephosphorylation cycles[62]. By contrast, dynamin2 (DYN2) is constitutively active and responsible for ubiquitous and constitutive endocytosis in all cell types[62-66]. Increasing evidence, however, indicates that DYN1 can regulate CME also in non-neuronal cells, and is selectively recruited to subsets of clathrin-coated pits, distinct to those containing DYN2 (refs. [66,67]). In these cells, similarly to neurons, DYN1 is kept inactive through GSK3β-dependent phosphorylation[36,65,68]. This inhibition is released upon EGF stimulation, leading to DYN1 activation. Thus, at variance with DYN2, DYN1 appears to be selectively activated by specific ligands. Interestingly, dynamins are deregulated in cancer[69]. DYN2 overexpression promotes invasiveness in different cancers, including hepatocellular carcinoma, prostate and pancreatic cancer[70-72]. DYN1 is aberrantly activated in non-small cell lung cancer where GSK3β function is inhibited, leading to dysregulated CME of the EGFR and to the acquisition of migratory/invasive properties (Figure 1e)[66,73]. In addition, DYN1 activation, via calcineurin-dependent dephosphorylation, upregulates endocytosis of death receptors, inhibiting apoptosis and contributing to cancer cell survival[74].

Thus, the specialization of the machinery employed in clathrin-coated pits — driven by the engagement of different cargoes, adaptor repertoire and cellular context (such as expression of particular components of the machinery or their differential regulation) — can impinge on cell behaviour in physiology and in cancer.

[H2] Context-dependent regulation of signalling by NCE and integration of outputs.

NCE comprises several internalization mechanisms differing in morphology of the internalizing structures at the plasma membrane, machinery, cargo and regulation[2,15,16]. Herein, we will not cover endocytosis through caveolae and refer the reader to extensive reviews on the subject[75,76].

The existence of so many NCE mechanisms might be, in part, due to inaccurate classification, as historically they were defined solely on the basis of clathrin-independence. Indeed – as we gain a deeper understanding of the specific features of NCE mechanisms, their overlapping characteristics and dependency on cellular context – it is becoming clear that some of them might represent "variations on a theme", rather than truly distinct processes (Figure 2).

CLIC/GEEC (clathrin-independent carrier/GPI-anchored protein-enriched early endosomal compartment) [77-79] is an NCE mechanism of central relevance: it relies on pleomorphic tubular endocytic intermediates and displays high endocytic capacity (Figure 2a)[2], rendering it suitable for mediating large plasma membrane rearrangements. CLIC/GEEC is thus implicated in plasma membrane turnover during cell spreading and migration[80] and in the regulation of membrane tension in adherent cells[81]. Indeed, upon a sudden reduction in plasma membrane tension, CLIC/GEEC is transiently activated to remove excess "floppy" membrane invaginations, thereby restoring the initial plasma membrane tensile state[79,81]. The opposite is also true: perturbation of CLIC/GEEC directly decreases plasma membrane tension. In this circuitry, vinculin acts as the CLIC/GEEC-sensing mechanotransducer at focal adhesions[81,82]. The mechanical buffering role of CLIC/GEEC differs from that of caveolae, which passively buffer the increase in tension also in non-adherent cells and seem to be critical for anchorage-independent growth[83]. An interesting view of CLIC/GEEC is that of a prototype coatless NCE mechanism mediated by a conserved core of molecular components, while other NCE routes might represent specialized variants. For instance, fast endophilin-mediated endocytosis (FEME)[84] has many commonalities with CLIC/GEEC[85], but differs in that it is strictly regulated by receptor activation and displays rapid kinetics (Figure 2b)[84].

Another NCE mechanism, crucial to the fate and signalling of its cargo, is EGFR-NCE[56,86]. This route internalizes the EGFR at high EGF doses in cellular contexts distinct from those where FEME and CLIC/GEEC are active (Figure 2c)[86,87]. EGFR-NCE relies on contacts between the plasma membrane and the endoplasmic reticulum (ER), which might generate the pulling force for the formation of plasma membrane tubular invaginations. These contacts are also necessary for localized Ca^{2+} release from the ER, required for NCE vesicle fission[87]. Since this route targets the EGFR to degradation and ultimately restricts its downstream signalling[86,87], it could represent a tumour suppressor pathway, as EGFR is commonly upregulated in cancer[88,89]**.** Of note, a preferential degradative fate for internalized receptors might characterize other NCE mechanisms, as shown – for instance – in the case of the β and γ subunits of the IL2 receptor (Figure 2d)[90,91]. However, it is worth pointing out that the degradative route does not necessarily preclude signalling from the internalized receptor, as shown for EGFR-NCE (see subsection Endomembrane dynamics controls collective motion).

Similarly to the EGFR, other RTKs are internalized via different endocytic routes. This is the case of MET (the receptor for hepatocyte growth factor), PDGFR (platelet-

derived growth factor receptor), VEGFR2, IGF1R (insulin-like growth factor 1 receptor) and NGFR (nerve growth factor receptor)[2,85]. For instance, IGF1R enters the cell via CME or through caveolae depending on the dose of IGF1 (refs. [92,93]). Similarly, depending on ligand concentrations, PDGFRB switches between CME and the CLIC/GEEC pathway[94-96]. These RTKs are also internalized via FEME[84]. Also in these cases, the choice of entry route might specify the signalling outcome, as exemplified by the VEGFR2 system[84,97-99]. Postnatal retinal angiogenesis is controlled by spatially regulated VEGFR2 endocytosis: mature vessels in the central retina display slow VEGFR2 endocytosis, while, at the edge of the growing vasculature, tip cells show high internalization rate, promoting the extension of the vessel network. In these cells, VEGFR2 is internalized both via ephrin-B2/DAB2-mediated CME and endophilin-A2-mediated NCE (resembling the FEME pathway). Consequently, CME of VEGFR2 promotes ERK signalling and vessel sprouting, whereas NCE regulates the signaling effector PAK, front–rear polarization, and migration of tip cells[98-100].

Macropinocytosis is a form of bulk NCE, which internalizes extracellular fluids and macromolecules in large heterogeneous vesicles (Figure 2e)[101]. In mammalian cells, it displays remarkable cell-specific variations in terms of molecular mechanism, fate of the macropinosomes (recycled or degraded), and regulation[102]. In macrophages and immature dendritic cells, macropinocytosis is constitutively active and critical for antigen presentation[102]. Conversely, epithelial cells show barely detectable levels of macropinocytosis in normal growing conditions. However, micropinocytosis is induced in epithelia by growth factors and pathogens, and is upregulated in cancer[102] by activation of oncogenes, such as RAS and SRC[103-105]. Under nutrient-deprived conditions, when the supply from the vasculature is insufficient, cancer cells can scavenge nutrients (mainly albumin) from the extracellular environment by macropinocytosis[106]. This provides a sufficient intracellular pool of amino acids (in particular glutamine) to meet the demands for energy production and nucleotide biosynthesis.

Through macropinocytosis, cancer cells can also internalize necrotic debris (which probably provides a higher quality source of nutrients), in a process called necrocytosis. This debris is degraded, providing amino acids, fatty acids, sugars and nucleotides used in anabolic pathways[107,108]. In prostate cancer cells, in nutrient deprivation conditions, necrocytosis is stimulated by PTEN loss, which cooperates with active AMPK to stimulate RAC1 -dependent macropinocytosis. AMPK is also required for RAS-driven macropinocytosis under nutrient restriction, indicating a general role of this kinase in macropinosome formation[107]. Context is again at play, since cancer cell lines displaying similar oncogenic alterations vary in the extent and modality (constitutive or inducible) of necrocytosis[108]. Importantly, necrocytosis promotes therapy resistance as it relieves stress induced by drugs targeting anabolic pathways[108]; thus, its inhibition could be exploited to target therapy resistant tumours[109].

[H2] Context-dependent role of endosomes in signalling and cellular responses.

Although the activity of signalling receptors starts at the plasma membrane, it is now abundantly clear that it persists in the various endocytic routes. In particular, endosomes are critical signalling ‘stations’ that: sustain signalling originating from the plasma membrane; are able to direct signalling through the recruitment of specific

effector molecules; create membrane microdomains where receptors are sequestered, upon specific post-translational modifications, and sorted to their final fate, thereby regulating signalling outcomes[5,110]. The centrality of endosomes as signalling platforms has been established for several signalling pathways in physiology and in cancer[1,5]. Here, we will focus on examples relevant to the idea of context-dependency.

GPCRs induce signalling from multiple cellular locations[111,112]. At the plasma membrane, GPCRs signal through the so-called "canonical pathway", leading to adenylyl cyclase activation and cAMP production. Upon prolonged ligand exposure, β-arrestins bind to phosphorylated GPCRs, extinguishing the signals and inducing receptor internalization (Figure 1c). β-arrestins also promote "non-canonical" GPCR signalling by their scaffolding function and activation of signalling pathways centred on ERK, p38 and JNK[111]. Canonical and non-canonical GPCR signalling can occur at the plasma membrane and from the endosomal station[111,112], in a context-dependent manner (see subsection Differential regulation of signalling by distinct clathrin-coated structures) and further demonstrated by in vivo studies of *Drosophila melanogaster* gastrulation, where tissue level regulation of GPCR endocytosis by specific factors and the dynamic partitioning of active receptors in different plasma membrane compartments, *i.e.,* flat plasma membrane or invaginations, determine different patterns of myosin II activation in mesodermal *vs*. ectodermal cells leading to differential tissue specification[113,114]. In addition, for some GPCRs, signalling was shown to be transmitted also from the Golgi compartment[115,116] and from the nucleus[117], regulating pathways distinct from those activated at the plasma membrane. Finally, adenylyl cyclases themselves are regulated by trafficking and localize to different cellular compartments, thus representing an additional variable for the diversification of GPCR signalling[118-121]. The emerging picture is that of a complex pattern of regulation, orchestrated by "localization biases" (*i.e*., the cellular compartment from which the signal originates) and dependent on cellular context, where different membrane compositions and specific sets of adaptors/interactors determine biological output[122-124]. This has enormous therapeutic implications. GPCRs are easily druggable: approximately 35% of all approved drugs are directed against them[125]. Further advances in this field will largely depend on knowledge of spatial resolution of GPCR signalling, to design more effective drugs with localization bias that would target a specific signalling output[126]. For instance, endosomal signalling of GPCRs has been involved in chronic inflammation and pain, and revealed to be an effective therapeutic target through the use of endosomally-directed GPCR antagonists[127-129]. These types of drugs may also benefit cancer patients; this is a particularly urgent need since, despite extensive knowledge of altered GPCR signalling[130-132] in tumours, only a few anti-GPCR drugs are approved for therapy in this disease[133].

Similarly, EGFR signalling is transmitted and regulated both at the plasma membrane and from endosomes, where specific signalling platforms are assembled to sustain and/or specify the signalling output (Figure 2c)[1,5]. A clear example of this regulation is represented by ERK1/2 signalling, which is involved in multiple cellular outputs, including proliferation, migration and survival[134,135]. Waves of ERK activation were observed in epithelial cell sheets during wound healing[136] and their propagation serves as a directional cue for collective cell migration[137]. This might be due to endosome-originated signals, as sustained ERK1/2 activation from endosomes is

necessary to exert a productive collective migratory response in breast epithelial cells[7,138]. Of note, in this instance, the EGFR is internalized through NCE-EGFR, a cell-specific, and hence context-dependent, process (see subsection Context-dependent regulation of signalling by NCE and integration of outputs). We will further discuss these issues later (subsection Endomembrane dynamics controls collective motion).

In addition to the recruitment of endosomal signalling effectors, the modulation of early endosome homotypic fusion represents another mechanism that regulates EGFR signal amplitude and duration, and thereby cellular response. This is achieved by the direct regulation of the fusion–fission machinery exerted by active EGFRs, leading to modulation of the number of endosomes, which may act as quanta signalling platforms that contain a pre-set number of activated receptors, thereby ultimately determining context-dependent programmes driving cell proliferation versus cell differentiation[139].

Finally, it is well established that endosomal membrane compartmentalization, that is, the specification of specialized membrane microdomains on the limiting membrane of endosomes, has a crucial role in receptor sorting and fate at endosomal signalling stations[110,140] with impact on the duration and specificity of signalling outputs. RAB GTPases are pivotal in this process, as they contribute to the definition of endosomal microdomains[141,142]. An example of how RABs define endosomal microdomains was elucidated studying RAB5 (refs. [141,143]): active RAB5, initially recruited to endosomes, can further recruit its own GEF, creating a local, positive feedback loop of activation. In addition, RAB5 directly modifies the local lipid environment stabilizing its own membrane recruitment, thus providing another level of positive feedback regulation that determines its patterning[144-149]. Notably, three highly conserved RAB5-encoding genes exist, which share biochemical and biological activity. However, they can also display specific functions depending on the cell context (as a function of their relative levels of expression[150]), signalling pathways that they impinge upon, and possibly subcellular localization[151-153]. Additionally, only the expression of RAB5A, but not RAB5B or RAB5C, is elevated in breast carcinomas and is capable of reawakening cell locomotion of jammed epithelial monolayers of breast tissue cells by differentially impacting on endosomal signalling (see section Endomembrane dynamics controls collective motion below).

A similar mechanism has been described for specification of endosomal microdomains by the small GTPase CDC42 (refs. [154,155]) and for organization of plasma membrane microdomains by K-RAS[156]. Such non-linear dynamics of recruitment and activation are typical of self-organizing systems that form spatial patterns on membranes[157], possibly representing a general mechanism for spatial organization of GTPases inside cells and for their role in generating membrane microdomains critical for receptor fate and sorting along the endocytic pathway[158].

[H1] Endocytosis in tissue morphogenesis

In this and in the following section, we will discuss the role of endocytosis and endocytic trafficking in the control of epithelial cellular collectives. In this section, we will highlight emerging principles relative to the role of endocytosis in tissue organization by focusing on the establishment of apical-basal cell polarity during

lumenogenesis of epithelial sheets, and tissue extension and cell shape morphogenesis in embryo development and adult epithelia.

[H2] Polarized transport controls apical-basal cell polarity and lumenogenesis

A defining feature of epithelial and glandular tissues is a pronounced apical–basal asymmetry, which is important for establishing barrier function, polarized transport[159,160], and successful lumenogenesis/tubulogenesis that gives these tissues their shape[161,162]. Failure of these mechanisms results in altered epithelial function, dysmorphologies and malignant transformation[163].

Directed membrane trafficking is critical for the polarized distribution of molecules in the cell and for the generation of specialized membranous domains in individual epithelial cells, but also for the coordination of epithelial polarization during lumen formation and the morphogenesis of glandular tissues[160-162]. There is extensive interplay between membrane trafficking and cell polarity (illustrated in Figure 3), whereby epithelial polarization involves not only the formation of apical and basolateral domains, but a whole topological re-organization of intracellular trafficking pathways along the apical-basal axis.

Two major membrane routes are used to establish apical-basal polarity in individual cells[164]. The first one involves recycling of polarity molecules from the plasma membrane followed by their sorting and targeted delivery to a polarized surface. The second one involves regulated trafficking of newly synthesized polarity determinants. Both of these routes are accomplished by polarized membrane trafficking regulated by RAB GTPases[165], which are key regulators of membrane trafficking also in non-polarized cells; however, of the ~70 mammalian RABs, only a dozen has been specifically implicated in regulating apical-basal cell polarity[166]. Furthermore, while some RABs operate regardless of environmental conditions, others control apical-basal cell polarity solely during 3D morphogenetic events, but not in 2D, indicating context-dependency[166].

Central to the establishment and maintenance of apical-basal polarity is the correct topological distribution of membrane-associated apical-basal cell polarity complexes (Supplementary Box 1) and lipid domains, organelles, and cytoskeletal structures[167] (Figure 3). A detailed description of apical-basal cell polarity complexes, their interaction with trafficking components, and their routes, is outside the scope of this review, but several excellent reviews on this topic are available[168-170].

How these events, occurring on a cellular level, orchestrate the morphogenesis of tube-shaped and sac-shaped structures is the object of intense scrutiny. A model system that reproduces the early steps of apical lumen formation and expansion is MDCK cells in 3D culture[160,171]. Following cell division, individual MDCK cells remain connected by a midbody, which serves as the spatial instructive cue for the creation of an apical membrane initiation site, thus establishing the location of the nascent lumen (Figure 4a). This process involves multiple trafficking steps. The first establishes the structural identity of the apical membrane initiation site through the coordination and re-organization of microtubules and branched actin filaments, via RAC1 GTPase-WAVE pathway and the adhesion protein, cingulin. This latter protein binds the central spindle microtubules and is recruited to the apical membrane initiation site via a direct interaction with the midbody and the tight junction protein, ZO1. Cingulin then can activate Arp2/3 complex to promote branched polymerization, which in turn recruits

more ZO1 — and consequently more cingulin — installing a positive feedforward loop critical for reinforcing the apical localization of these proteins[172]. Cingulin also serves as a tethering platform for the anchoring of apical endosomal vesicles by directly interacting with FIP5, a RAB11-interacting protein[172,173] (Figure 4b). Consequently, transmembrane proteins, such as podocalyxin (a classical apical marker) and Crumbs polarity complex— which undergo transcytosis from the extracellular matrix abutting surface to the apical membrane initiation site via RAB11-marked endo/exocytic structures (which are most likely also positive for RAB8; see discussion in the following paragraph) — can be hooked to the apical membrane initiation site through interaction with cingulin and FIP5. Alternatively, association of apical polarity proteins with the apical membrane initiation site could occur through binding to RAB35, which in addition to cingulin has been proposed to act as an anchoring platform for polarity determinants[171,174]. Although the exact molecular role of RAB35 is still debated, there is a consensus that its function is important for the correct transport of apical and basolateral determinants[166,175].

Additionally, RAB11 can also activate RAB8 through the GEF RAB3IP (aka Rabin8) on Rab11 and RAB8 double-positive endosomes. Both RAB8 and RAB11 can I then recruit myosin motor, MYO5B and the EXOC6 (aka Sec15A) exocyst subunit in order to transport recycling vesicles that carry critical polarity determinants to the apical membrane initiation site[176], and they also interact with EXOC5 (aka Sec10) at the apical membrane initiation site to promote vesicle docking (Fig. 4B, inset)[171].

Finally, apical membrane initiation site is characterized by a unique phospholipid composition, being enriched in di-phosphorylated phospholipids, including PI(3,4)P2 (ref. [177]) and PI(4,5)P2 (ref. [178]). This lipid environment is critical for the asymmetric recruitment of annexin-2 and of the CDC42 apical polarity complex, which is in turn capable of binding key apical polarity determinants, PAR3 and PAR6[178]. These lipids also ensure the correct localization and function of membrane-curvature sensor proteins. One such protein, IRSP53 (aka BAIAP2), binds to PI(4,5)P2-rich membranes. In the early stages of cyst formation, IRSP53 re-localizes from the plasma membrane to endosomal structures and to the apical membrane initiation site, where, through its I-BAR domain (which binds to negatively curved membranes), it ensures the integrity and shape of the opposing membranes of the two neighbouring cells for correct lumen generation. Loss of IRSP53 results in the formation of aberrant cytoplasmic bridges that interconnect adjacent cells and interrupt the continuity of the nascent lumen, yielding aberrant multiple lumens[179].

It is still unclear how, after the establishment of the apical membrane initiation site, a lumen develops and expands. It is likely that a balance of forces between tensile junctional elements and cell–substrate adhesion, contributes to the position and shape of the de novo lumen[180,181]. Additionally, lumen opening might be driven by: reduced actomyosin contractility at adherens junctions, which allows fluctuation of the membrane at the interface between two adjacent cells; and/or by increased intraluminal hydrostatic pressure, which follows the establishment of tight junctions and the polarized flux of water through ion channels (Figure 4c). Notably, as the lumen inflates the cell volume decreases, suggesting that the system is isochoric and proceeds through volume conservation between the cells and the luminal space[182].

An invariable feature of carcinomas is the loss of epithelial stereotypical architecture. Observations in *D. melanogaster* identified polarity regulators as tumour

suppressors. By contrast, studies in mammals suggest oncogenic dominant roles of these proteins[183]. A number of polarity determinants – including PARD6B, SCRIB, PRKCI and DLGs – are amplified or display aberrant cellular distribution in cancer[183]. This suggests that carcinomas might exploit polarity proteins to promote their progression. For example, polarity proteins, when overexpressed, might switch from controlling apical-basal cell polarity to regulate subcellular polarity (intracellular asymmetry), which can improve cellular fitness to execute functions such as proliferation, apoptosis, stress adaptation, stemness and organelle biology[163]. Membrane trafficking processes are expected to be directly involved in the regulation of this polarity switch and may thus contribute to the onset and progression of carcinomas.

[H2] Tissue organization by mechanosensitive membrane trafficking.

At variance with apical-basal cell polarity, planar cell polarity is a system of information that provides cues along the axis parallel to the epithelial plane, and requires the establishment of asymmetry within individual cells and the alignment of these asymmetries across dozens or hundreds of cells. This is achieved through the proximal-to-distal distribution, along the plane, of six planar cell polarity proteins (Supplementary Box 1)[184-187]. The planar distribution of these proteins is driven by a series of feedback loops, typically operative at the single cell level and dependent on endocytosis and polarized membrane trafficking, as described in recent reviews[188,189]. Here, we will focus on additional, more recently described, endocytic mechanisms that respond to tissue mechanics and control individual cell shape in the context of supracellular, tissue-level organization.

One such mechanism involves the control of cell geometry and of the actomyosin force-generation machinery, which regulate tissue rearrangements and size, as elucidated in several developmental systems[190], including germband extension in *D. melanogaster*[191,192]. Here, tissues undergo elongation to attain their final shape through a process of cell intercalation that drives the so-called convergent extension — a cell rearrangement whereby cells intercalate in an oriented fashion causing tissues to shrink along the dorso-ventral axis and to elongate along the antero-posterior axis (Figure 5a). At the level of neighbouring cells, dorso-ventral intercalation is the result of the contraction of the interface between two dorso-ventral neighbours, followed by the extension of the interface between the antero-posterior neighbours[193]. This occurs through the combination of a tension-producing actomyosin network and adherens junctions, which are governed by spatially restricted membrane trafficking, specifically occurring along the surface of neighbouring cells[194]. Indeed, during germband extension in *D. melanogaster*, clathrin and AP2 become enriched at the contractile interface of neighbouring cells in the antero-posterior direction[195]; here, polarized actomyosin contractility drives clustering and internalization of E-Cadherin, which leads to junctional shrinkage by reducing local cell–cell adhesion[195].

The interplay between actomyosin contractility and endocytosis goes beyond regulation of planar cell polarity, impinging also on the homeostatic control of tension state and cell size in tissues, as occurs during the contraction of amnioserosa during dorsal closure in *D. melanogaster*[196], and in adherens junction remodelling during morphogenetic cell shape changes in mammalian epithelial tissues[197]. In the former example (Figure 5b), adherens junctions tune their length and maintain junctional straightness and tension while the whole tissue undergoes a global contraction[198].

When the tissue contracts, junctions shorten resulting in excess plasma membrane that folds inwards, causing a reduction in membrane tension. Junctional and membrane tension is restored by promoting removal of excess or folded plasma membrane at junctions, through endocytosis[198]. The opposite occurs when junctions are stretched, which results in the redistribution of actomyosin to adherens junction for reinforcement, which increases tension that is then restored through inhibition of endocytosis. Similarly, in various mammalian epithelial monolayers, endocytic pathways act as mechanosensitive networks to stabilize junctional tension and length. In these systems, junctions are dynamically remodelled by short pulses of RHOA-mediated actomyosin contractility[197]. For pulses of RHOA activation that induce a contraction capable of overcoming a defined strain threshold (*i.e.*, the maximal deformation that epithelial cells within an epithelial tissue can undergo without rupturing) associated with morphogenetic tissue shaping, junctions are permanently relaxed and subsequently shortened in a process that requires endocytosis[197].

These findings highlight that changes in cell and tissue size are frequently driven by pulsatile processes, where a directed dynamic process (*e.g.*, shortening of a junction) alternates with periods in which force-generating networks are restored. This cycling requires consolidation or "ratcheting" to lead to permanent changes, by preventing the reversal of cell shape once the contractile network has dissipated. Membrane trafficking operates as such a ratchet to ensure the removal of slackened membrane, as mediated by RAB35 in germband extension[199,200]. At the antero-posterior interface, RAB35 accumulates in compartments that dynamically grow and shrink on the minute timescale. Genetic removal of RAB35 does not prevent the contractile cycles of junctional length but impedes the permanent shortening of the cell interface (Figure 5b).

It is noteworthy that trafficking regulators, such as RAB35, appear to play multiple roles in controlling the proper abscission of dividing cells[201] and integrin trafficking during glandular morphogenesis[166], in addition to their role in tissue adaptation to mechanical stresses. Indeed, the ability of tissues to adapt and respond to mechanical perturbations has emerged as a key mechanism to ensure proper organ and organismal development; whereas, failure to mount this mechanoresponse can lead to patterning and structural abnormalities in the developing embryo. RAB35 also controls the ability of cells to internalize nutrients through macropinocytosis[202] and was identified as a pro-oncogenic mutated RAB capable of interacting with the regulatory subunit of PI3K (p85) — a key oncogene — enhancing the activity of this kinase[203]. How this pro-oncogenic function of RAB35 impinges on glandular morphogenesis and tissue mechanoadaptation is unknown, but it is tempting to speculate that RAB35 might be at the centre of a trafficking network that coordinates tissue shape, division and growth, thus directly influencing tumorigenesis when its activity is perturbed[204].

[H1] Endocytosis in collective cell migration

One of the best characterized functions of endocytosis, beyond simply uptake of extracellular cargo, is in the regulation of cell migration in physiology and in invasive cell migration in cancer, as discussed in recent reviews[18, 205-207]. In this section, we will focus on the regulation of cell migration by endocytosis, in the context of collective modalities of migration with emphasis to their subversion in cancer (see also

Supplementary Table 2). In particular, we will discuss three emerging aspects of endocytic regulation of collective migration: promoting persistent migration of cell monolayers, modulation of tissue mechanical and material-like properties and induction of EMT.

[H2] Endomembrane dynamics controls collective motion.

A function of endocytosis is the coordination of soluble and mechanical cues during individual cell migration[7,10,208-210]. Frequently, however, cells are embedded in multicellular ensembles, where the motion of the whole cell collective is overarching with respect to individual cell locomotion. This occurs during the development of epithelial layers (skin, digestive or respiratory tracts), in glandular organs, or during wound healing. Collective motion may also take the form of cell clusters that maintain cohesive interactions while migrating, as in the case of border cell migration in *D. melanogaster*[211] or during the dissemination of epithelial cancers[212,213]. In these situations, individual cells must coordinate their movement with that of their neighbours, while keeping tight cell–cell interactions.

Multicellular migration is ruled by the biochemical and physical interactions that cells establish with one another and their environment[214]. Indeed, physical forces exerted by individual cells on their substrate or propagated, long-range, in multicellular cohorts through cell–cell adhesions (via cadherins at adherens junctions), are principal determinants of multicellular dynamics[215]. Establishing and maintaining adherens junction strength, dynamics and polarity during cell migration is achieved by trafficking membrane cadherins. One example here is collective migration of astrocytes — major glial cells of the central nervous system — during development and wounding[216]. Astrocytes migrate collectively through maintenance of connections via N-Cadherin (aka CDH2). Developmental or wounding cues induce polarization of astrocyte monolayer with the establishment of “leader cells” that determine the migratory front. These leader cells extend lamellipodia, where actin treadmilling and actomyosin contractility generate a retrograde flow of actin. N-Cadherin travels backwards along this flow (retrograde transport along the plasma membrane). At the back, N-Cadherin is endocytosed and through polarized trafficking delivered to the front, to form new junctions[216], virtually installing a membrane trafficking treadmilling process for N-Cadherin (Figure 6a), which can support persistent and cohesive migration of the entire cell sheet.

Another example is the collective motion of the neural crest in *Xenopus laevis*[217], where cells become fully migratory before they complete cell–cell dissociation, thereby moving as collective clusters. A signalling mechanism, affecting junctional adhesion strength, is triggered by lysophosphatidic acid (LPA)[218]. In the neural crest, the interaction of LPA with its receptor, LPAR, specifically affects collective motion by modulating the extent of cell–cell cohesion through internalization of N-Cadherin[219]. Neural crest collectives undergo a transition from a solid-like state, where local cell rearrangements (the motion of a cell with respect to its neighbours) are not permitted, to a fluid-like state, where reduced Cadherin adhesion allows local cell rearrangement[219]. This results in fluidization of neural crest clusters, which can more easily migrate into confined spaces, while retaining cell–cell cohesion.

LPA is a signalling lipid, abundant in blood, skin and ascitic fluids, which acts both as mitogen and chemotactic agent. Melanoma and pancreatic cancer cells can

611 breakdown LPA, generating outward-facing local gradients — low in the tumour and
612 high in the surrounding environment — that guide metastatic cell dispersal through a
613 self-generated LPA chemotactic gradient[220,221]. Engagement of the LPAR stimulates its
614 endocytosis in early endosomes. N-WASP, a promoter of branched actin
615 polymerization, together with the endocytic protein SNX18, coordinate recycling of
616 internalized LPAR from RAB11–RAB8-double positive endocytic tubules back to the
617 plasma membrane preventing its degradation. The N-WASP-mediated LPAR recycling
618 then ensures constant replenishment of the receptor at the plasma membrane
619 allowing persistent RHOA activation, resulting in increased cell contractility, generation
620 of traction forces and matrix remodelling for efficient cell migration. Loss of N-WASP
621 affects invasiveness of pancreatic cancer cells pointing to N-WASP-dependent
622 chemotaxis to LPA gradients as a motivator of egress from primary tumours and
623 tropism towards metastatic sites[221]. In HeLa cells, the hyperactivation of the LPAR
624 might also occur through trapping the receptor at the plasma membrane in coated
625 plaques[222] (Box 1): reduced LPAR internalization prolongs its downstream signalling,
626 representing an alternative mechanism to recycling for regulating actomyosin-based
627 contractility. Whether coated plaques are operative in pancreatic cancer cells remains
628 an issue for future investigations; notably, these structures are increased in cells plated
629 on stiff substrates[33], suggesting that the hyper-stiff, stromal-rich, microenvironment of
630 pancreatic cancer cells might favour their formation.
631 These findings highlight how an integrated biochemical and physical perspective is
632 necessary for a holistic comprehension of the dynamics of multicellular entities and
633 tissues. Such dynamics can be better understood by considering cell collectives as
634 ensembles of “active particulate matter” that are, nevertheless, governed by structural
635 and dynamic physical properties typical of amorphous viscoelastic materials[223,224].
636 During tissue growth, cells are free to move, as in a fluid, but their motion becomes
637 constrained as cell density increases. At a critical cell density, motility ceases and
638 collectives rigidify undergoing a liquid-like to solid-like transition referred to as tissue
639 jamming[225-229]. This transition is thought to promote the development of elasticity and
640 of barrier properties, but also to act as a suppressive mechanism for the growth of
641 oncogenic clones.
642 Recently, it was shown that global perturbations of endocytosis — achieved by
643 modulating the master regulator of early endosome biogenesis, RAB5A — impinges on
644 biomechanical properties of cellular collectives. Increased RAB5A levels were sufficient
645 to re-awaken the motility of “jammed” epithelial monolayers by triggering millimetre-
646 scale, coherent and ballistic locomotion of multicellular streams that flow like
647 “flocking” fluids[230], through the modulation of EGFR signalling (Figure 6b and 6c). In
648 mammary epithelial cells, RAB5A induced EGFR-NCE, leading to accumulation of
649 activated receptors in endosomes, whose size and number were remarkably
650 elevated[231], and to elevation of endosomal ERK1/2 signalling, in keeping with the
651 concept that endosomes act as quanta-like platforms where phosphorylated EGFR can
652 be packaged at constant mean amounts[139]. In turn, this caused the hyper-
653 phosphorylation of the Arp2/3 nucleating promoting factor, WAVE2, fuelling the
654 extension of lamellipodia and directed cell motility[231] (Figure 6b).
655 Tumour cells can exploit this endocytic-mediated regulation of cell mechanics to
656 facilitate their collective dissemination. Consistently, mammary tumour spheroids
657 overexpressing RAB5 display persistent angular rotation and elevated local cell

rearrangements[231]. This leads to the generation of large traction forces on the surrounding extracellular matrix, resulting in its remodelling and the generation of tracks and channels into which tumour cells invade collectively. In another study, lowering of cell–cell adhesion with concomitant increase in 3D matrix confinement have been shown to affect unjamming transition and to foster collective invasion in breast cancer models[232]. These dynamics are reminiscent of those during body axis elongation in the zebrafish embryo, where a gradual solid-like to fluid-like transition is critical for promoting the elongation of the entire body axis[233]. Within this context, membrane trafficking might be a general mechanism to control the transition from a solid-like/jammed to a liquid-like/unjammed state, leading to progressive fluidization and collective motion.

[H2] Endocytosis and EMT

In addition to solid-to-liquid transition, another process contributing to collective motion of tissues is EMT. Unlike solid–liquid transitions, however, which rely on mechanical remodelling, EMT primarily involves changes of individual cell identity and state. Accordingly, recent evidence established that unjamming and EMT are very distinct processes differing not only in molecular machinery, but also in physical, dynamic, geometrical and structural properties[234]. Indeed, unjamming and EMT might be viewed as complementary gateways to cell migration, driving the escape of epithelial cells from a static, rigid and sessile state.

EMT is a process in which cells shift from a sessile epithelial to a migratory/invasive mesenchymal-like state[235]. Physiologically, it is involved in morphogenetic events, including gastrulation and neural crest migration[236]. Pathologically, EMT is exploited by cancer cells to acquire invasive/metastatic ability[237-239] — a process also connected with the acquisition of a cancer stem cell-like phenotype and drug resistance[240-243].

Various mechanisms act as initiators of EMT in cancer, including: growth factors and cytokines, components of the extracellular matrix and mechanical cues. Given the involvement of endocytosis in cell response to all these factors, it is not surprising that it controls EMT, at multiple steps in the process [244,245]. The common distal effectors, on which all these signalling pathways converge, are a set of transcription factors, which include Snail, Slug and ZEB1/2[246-248]. These transcription factors orchestrate the transcriptional repression of epithelial-specific genes that are critical in cell adhesion (*e.g.*, E-Cadherin), and the transcriptional activation of mesenchymal genes that reshape the cytoskeleton and the plasma membrane to permit migration (*e.g.*, N-Cadherin and vimentin). Thus, the actual enactment of the EMT program is generally viewed as a transcriptionally-driven process. This view is changing.

The key to understanding this renewed vision is the finding that, at least in some cases, EMT is not a binary process involving cell state conversion, but rather involves a plastic state in which cells can exhibit intermediate phenotypes, retaining some epithelial characteristics while acquiring some mesenchymal ones[236,237]. This state, called partial or plastic EMT (P-EMT), appears to be advantageous for cancer cells to oscillate between a mesenchymal phenotype, necessary to migrate out of the primary tumor, and an epithelial identity, necessary for survival and proliferation once they reach the final metastatic niche[249]. Cell context is emerging as a determining factor for the decision to adopt a full EMT *vs*. a P-EMT state: in this latter instance, endocytic mechanisms appear paramount over transcriptional ones. Cancer cells adopting the

"endocytic" modality to achieve P-EMT, lose their epithelial phenotype through surface protein internalization rather than through the action of EMT-promoting transcription factors[250]. In particular, E-Cadherin is actively internalized and sequestered in RAB11-positive recycling endosomes, consistent with the notion that the molecular alteration behind the cell state shift resides in a blockade of the physiological recycling of E-Cadherin to the plasma membrane.

In this contention, we note that the "endocytic mechanism" of P-EMT has the characteristics of being more rapidly reversible (through re-induction of recycling) and perhaps less extensive than the transcriptional reprogramming, thus being more adapt to the plastic state of P-EMT. It is also interesting that cells adopting the endocytic-based P-EMT modality, exhibit a collective, rather than individual, mode of migration, characteristic of cells displaying full EMT[250]. Furthermore, it has been shown that cancer cells adopting different degrees of EMT, tend to metastasize to different sites[251] raising the interesting question of whether a primary alteration of endocytic mechanisms, in some tumours, might underlie metastatic organotropism.

[H1] Conclusions and perspectives

Since the first hints, more than a quarter of century ago, that endocytosis could sustain and/or diversify cell signalling[252-254], rather than merely extinguishing it, a wealth of literature has accumulated, which not only supports this concept but extends it to virtually every area of cell regulation[1,255]. The field is now mature to address the next question: that of the significance of cellular context of endocytic regulation.

In this vision, the same signalling circuitry could elicit substantially different biological outputs based on the "endocytic landscape" in which it is embedded and regulated by. Although the concept of the existence of specific endocytic landscapes in cells, herein proposed, is at present molecularly vague, we would like to draw one example that may support it. The EGFR is internalized by two types of CME, by FEME and by EGFR-NCE, which all impact differently on receptor fate, signalling properties, and timing of signals (see section Endocytic regulation in context). They also display cell context specificity. Thus, cell-specific "endocytic landscapes" are projected to differentially affect the EGFR signalling output — an issue of great pathophysiological relevance, given the importance of this RTK to cancer[88,89].

There are clever approaches being pursued to investigate this hypothesis. One is that of exploiting isogenic models of cells differentiated in vitro from multipotent cells (embryonic stem cells or induced pluripotent cells). Using this approach, it was shown that isogenic, diversely differentiated cells display remarkable variations in the dynamics and structure of clathrin coats[40]. These differences hinged on context-dependent variations in AP2 levels and in the requirement for PI3K activity[40]. Another approach is to employ organoids that recapitulate the complex differentiation patterns that occur in vivo[256]. This approach, coupled to advanced imaging to detect live endocytic dynamics and to single-cell sequencing, to decipher the endocytic landscape of individual cells, should substantially improve our understanding of cell context in individual and collective cellular behaviour.

The same view could be extended to the domain of endocytic alterations in cancer. In this case, perhaps, the simple idea of endocytic proteins acting as oncogenes or tumour suppressors, albeit valid in some cases[1,17,18], might be too narrow. The

evidence that the endocytic context might skew the output of a signalling pathway towards different biological outcomes, opens up the possibility that differences in the expression of groups of endocytic proteins (the "endocytic landscape") might render cells more or less prone to respond to oncogenic insults and, therefore affect the propensity of cells for tumorigenic transformation. In this contention, we note that genetic alterations of endocytic proteins are infrequent in cancer[1,17,18]; rather, the more common alteration is their over- or under-expression, which often correlates with metastasis and disease outcome[6,18] (see also Supplementary Tables 2 and 3) To extend our knowledge, we should go beyond the "one protein at the time" approach and embrace comprehensive profiling of tumours, to identify endocytic landscapes that might define the propensity of certain tumors (or subgroups of tumours) to adopt different individual or collective cell behaviours (even in response to the same signalling input), especially those relevant to the invasive/metastatic phenotype.

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Competing interests
The authors declare no competing interests.

Figure Legends

Figure 1. Heterogeneity of clathrin-coated pits.
Clathrin-mediated endocytosis (CME) is a heterogeneous process, with a variety of clathrin-coated pits (CCPs) that differ in their composition, leading to different fates of the endocytic cargo. **a**. In physiological conditions, transferrin (Tf) receptor (TfR) internalization depends on clathrin adaptor AP2 only[48-51]. After endocytosis, the iron bound to Tf is released in an endosomal compartment and the Tf–TfR complex is recycled back to the surface[257]. **b**. Low-density lipoprotein (LDL) receptor (LDLR) endocytosis relies on the cargo-specific adaptors, DAB2 and ARH, in addition to AP2 (refs. [258-263]). In the endosomal compartment, the LDL moiety is released and committed to lysosomal degradation (and the LDL-bound cholesterol is released),

while the "empty" receptor is recycled to the cell surface[264]. **c**. Agonist-activated G protein-coupled receptors (GPCRs) bind heterotrimeric G-proteins (α, β, γ) which triggers "canonical" G-protein-mediated signalling at the plasma membrane[111]. Receptor desensitization occurs by phosphorylation (P) of the active receptor by GPCR kinases (GRKs) and subsequent binding of β-arrestin, resulting in dissociation of the G-protein complex and of its interaction with the receptor. Further desensitization occurs when the β-arrestin bound GPCR is sequestered into distinct CCPs and internalized, to be either recycled back to the plasma membrane or degraded in the lysosome (not shown)[41]. Receptor ubiquitylation (Ub) and PDZ-containing scaffold proteins, linking the GPCR to the actin cytoskeleton, determine increased surface retention of GPCR-containing CCPs and their slower kinetics, promoting cargo clustering and β-arrestin-dependent "non-canonical" signalling[41-44]. **d**. Epidermal growth factor receptor (EGFR) can be internalized through different CCPs[51]: AP2-dependent CCPs mostly commit the receptor to recycling, sustaining signalling; AP2-independent CCPs, which rely on the EPS15 and/or EPS15L1 and EPN1 adaptors, target the EGFR to lysosomal degradation[51]. **e**. Dynamin2 (DYN2) is ubiquitously and constitutively involved in CCP maturation and fission (parts a-d)[62-66]. By contrast, dynamin1 (DYN1) is employed in clathrin-mediated endocytosis only under specific conditions; *e.g.*, in EGF stimulated non-small cell lung cancer cells where the inhibitory constraints of GSK3β-dependent DYN1 phosphorylation are removed[36,65,68]. In cancer, the GSK3β kinase can be inactivated (not shown) or targeted for degradation due to aberrant activation of AKT kinase, leading to DYN1 dephosphorylation and aberrant activation, and eventually to deregulated EGFR endocytosis, signalling and migration[66,73].

Figure 2. NCE mechanisms and cellular contexts.

a. CLIC/GEEC (clathrin-independent carrier/GPI-anchored protein-enriched early endosomal compartment) requires extracellular clustering of glycosylated cargoes (through galectins) to induce membrane bending, and small GTPases (ARF1, CDC42) that coordinate the activation of actin nucleators (Arp2/3) and regulators (NWASP)[2,15]. Bar-domain proteins (GRAF1, IRSP53, PICK1) control the initial phase of vesicle formation, while endophilinA2 (EndoA2) is required for fission, together with the pulling force of the actin cytoskeleton and the microtubule-associated motor protein, dynein: a mechanism known as "friction-mediated scission"[265,266]. The internalized vesicle fuses with an endosomal GEEC compartment from which cargoes are recycled[2,15]. **b**. Fast endophilin-mediated endocytosis (FEME), which is active in the same cells as CLIC/GEEC[85], also requires the action of actin, actin nucleators, and BAR-domain proteins. EndoA2 is required for vesicle formation and fission (together with dynein, microtubules and DYN2)[85]. FEME internalizes epidermal growth factor receptor (EGFR) stimulated with high EGF, and other cargoes, including other receptor tyrosine kinases (RTKs) and G protein-coupled receptors (GPCRs)[84]. **c**. High EGF also induces EGFR-NCE, which requires EGFR ubiquitylation (Ub) and Ub receptors (EPS15, EPS15L1 and EPN1)[56], plasma membrane–ER contacts mediated by the ER transmembrane protein RTN3, and Ca^{2+} release at plasma membrane–ER contacts (of note, some mechanistic aspects of EGFR-NCE are still missing, *e.g.*: Which are the factors involved in plasma membrane–ER tethering? Is RTN3 directly recruited to the EGFR? Which is the exact role of EGFR ubiquitylation and Ub receptors?)[87]. EGFR-dependent signalling occurs at the plasma membrane and in endosomes through ERK1/2 (refs. [139,231]).

Notably, activated ERK1/2 and EGFR might not accumulate on the same endosome after CME[267]. They might, instead, do so in cells expressing high EGFR and treated with high EGF concentrations, or expressing elevated RAB5A (ref. [231]) (see also Fig. 6), where EGFR-NCE and massive activation of pinocytotic processes occur[268]. Eventually, EGFR-NCE targets the EGFR to lysosomal degradation, restricting signalling in a cell context-dependent fashion[86,269]. **d**. The interleukin-2 (IL-2) receptor (IL-2R) complex (comprising α, β, subunits) is expressed in lymphocytes and signals through JAK–STAT[270]. IL-2R is internalized via an NCE mechanism occurring in proximity of actin-enriched membrane protrusions that facilitate actin polymerization and plasma membrane invagination[90,271-273]. Once internalized, the α chain is recycled, whereas the β and γ chains are degraded[91]. **e**. Macropinocytosis internalizes pathogens, apoptotic cells/debris and proteins through the formation of actin-dependent plasma membrane protrusions[101]. Internalized materials are delivered to the lysosome. In the lysosome, pathogen-derived antigens are generated and bind to MHC receptors (in immune cells), which are targeted to the plasma membrane for antigen presentation[102]. Apoptotic cells/debris and proteins are degraded in lysosomes and used by epithelial cells (particularly, cancer cells) for nutrient scavenging[103,107-109].

Figure 3. Membrane trafficking in apical-basal cell polarity.
The apical-basal polarity complexes (see Supplementary Box 1) are shown as green ovals, including their main protein components. Epithelial polarity is maintained through coordinated and polarized trafficking of the various components of these complexes, achieved through distinct endosomal intermediates, regulated by different RAB GTPases, as shown[274]. There is bidirectional connection between polarity complexes and membrane traffic. For instance, RAB11-dependent apical transport pathways reinforce the localization of the apical PAR complex, while apical PAR3, controls the subapical positioning of RAB11-positive endosomes[171,275].

A number of other apical and basolateral cargoes enter spatially distinct, peripherally localized apical or basolateral early endosomes (AEE or BEE), respectively, and undergo alternative fates[276]. They can be either recycled to the plasma membrane (not shown), or routed to RAB8—RAB10-double positive common recycling endosomes (CRE)[277,278]. CREs are so named because they are the target of both apical and basal cargoes, which are then either shuttled to the basolateral surface, or delivered to the apical recycling endosomal (ARE) compartments, before being directed to the apical surface[276]. For example, basolateral proteins – such as E-Cadherin or transferrin receptor – are targeted from CRE to the basolateral plasma membrane via a pathway that involves the AP1B coat protein as well as the exocyst protein complex (not shown)[279]. An example of an apical cargo that traffics via AREs to the apical plasma membrane is H^+/K^+-ATPase of gastric parietal cells[280]. Neosynthesized proteins can also be delivered, via Golgi, to their destinations (apical or basolateral) either directly (not shown) or indirectly through AREs or CREs. The green arrow points to the aPKC/LGL circuitry described in Supplementary Box 1.

Figure 4. Membrane trafficking in apical lumen formation.
Lumen formation in epithelial monolayers requires coordination between cell division, actin polymerization and membrane trafficking[160,171,281] (events are shown across two cells for clarity but they occur in both cells). **a**. Following cell division, at the midbody,

the apical membrane initiation site (dashed box) is established, through the coordination and re-organization of microtubules (not shown) and branched actin filaments, via RAC1—WAVE to promote recruitment of cingulin. A positive feedback loop (asterisk) involving branched actin polymerization, ZO1 and cingulin further sustains the establishment of the apical membrane initiation site (whereby cingulin promotes branched actin polymerization, which supports recruitment of ZO1 and consequently recruitment of cingulin via its interaction with ZO1)[172]. At this stage, early polarity determinants such as podocalyxin or IRSP53, are present on the plasma membrane facing the outer surface. **b**. Multiple endocytic and transport events (1) direct apical determinants (podocalyxin and IRSP53 are shown, but there are several others) to the apical membrane initiation site, where the exocytosis of membrane and apical proteins results in formation of a nascent lumen (2). IRSP53 senses and stabilizes the negative curvature of the membrane via its I-BAR domain, which ensures the integrity and shape of the opposing membranes of the two neighbouring cells for correct lumen generation. RAB11–RAB8-double positive endosomes are critical in this phase (3 and inset)[171], as they become restricted towards the luminal side, ideally positioned to dock and fuse at the apical membrane through multiple interactions. These endosomes can be tethered to the apical membrane initiation site by binding to cingulin (via FIP5–RAB11 (4)], by binding to RAB35 directly (5) or through tethering with the exocyst complex (including MYO5B, Sec15a and SEC10)[282] (6, inset). Among the cargoes transported by the RAB8–RAB11 vesicles, are annexin2 and CDC42, which are directed apically to interact with apical polarity complex component aPKC (7, inset; see also Supplementary Box 1 for details on epithelial polarity)[171]. Thus, a self-sustained polarized transport system of apical determinants is essential and precedes the formation of a tight junction-delimited lumen. **c**. Subsequent cell divisions expand the luminal space and increase the size of the luminal cyst through a combination of water influx through solute carriers that increases hydrostatic luminal pressure (blue arrows) and reduced actomyosin contractility (red arrows; the exact molecular mechanisms are unclear) along the forming tight and adherens junctions.

Figure 5. Endocytosis and actomyosin in the control of cell shape and tissue elongation.

A. Diagrammatic representation of cell intercalation in *Drosophila melanogaster* germband extension[193] (the arrows show the developmental axes: A, anterior; P, posterior; D, dorsal; V, ventral.). T1, T2, T3 indicate the different phases of the process. During the T1-to-T2 transition, the antero-posterior interface between two adjacent cells shrinks to a four-cell vertex (T2, which defines a state where the sides of four adjacent cells converged into a single focal point indicated with a red dot). This is thought to occur because of increased actomyosin medial contraction that generates forces directed along the antero-posterior plane (1). This event is accompanied by elevated and localized clathrin-mediated endocytosis (CME) of junctional molecules, such as E-Cadherin[193,195] (2). In the T2-to-T3 transition, the four-cell vertex expands to generate a new cell-junctional surface oriented along the dorso-ventral interface that leads to effective tissue elongation along the dorso-ventral axis. **b**. Contraction of amnioserosa in dorsal closure in *D. melanogaster*[196], and in morphogenetic junction remodelling in mammalian epithelial tissues[197]. In the initial step, changes in RHOA-mediated activity result in increased medial-apical actomyosin contractility (1) that

causes cell areas to oscillate leading to increased junctional tension (2). During area oscillations, RAB35 endocytic activity is enhanced resulting in increased endocytosis (3). This aims at reduction of plasma membrane tension. Concomitantly, internalization of the plasma membrane and various plasma membrane cargo proteins (*e.g.*, E-Cadherin) leads to shortening of the junctional interfaces[196] (4). Myosin II and CME are required to terminate the RAB35 intermediate compartment (not shown) and to direct endocytosed materials to endosomes. This process of internalization of the plasma membrane and junctional molecules functions as an effective ratcheting device to remove excess membrane and to maintain the reduced length of cell–cell junctions. However, through not yet fully understood mechanisms, cargoes – such as CDH1 – can be routed back from endosomes to the interface between the two cells (5), thereby replenishing the membrane and interfacial components and ensuring the reversibility of the interface contraction (6).

Figure 6: Endomembranes in the dynamics of collective motion.
a. The treadmilling cycle of N-Cadherin in developmental collective motion of astrocytes. Cells at the front show protrusive edges. F-actin attachment to adherens junction components (catenins and N-Cadherin) drives their retrograde flow (1) along lateral contacts (2). At the rear, phosphorylation (P) of p120-catenin by GSK3 untangles the complex (3) allowing for N-Cadherin endocytosis (4) and polarized recycling to the leading edge where GSK3 is inactive (5)[216]. **b**. In epithelial 2D and 3D collectives that depend on epidermal growth factor receptor (EGFR) activation for proliferation and motility, the elevation of RAB5A triggers non-clathrin endocytosis (NCE) of the activated EGFR leading to the concomitant expansion in the size and number of endosomes. This results in sustained ERK1/2 signalling from endosomes. ERK1/2 signalling promotes the phosphorylation of WAVE2 (WAVE2-P)[231], which, in the presence of activated RAC1, leads to the activation of Arp2/3 complex and branched actin nucleation[283,284] leading to the formation of "cryptic lamellipodia"[285]. These structures extend beneath neighbouring cells and drive persistent, coordinated cell motion and efficient cell re-orientation, which, together with elevated junctional tension (not shown), results in unjamming of the entire monolayer[138,231,286,287]. **c.** Snapshots representative of two distinct states of densely packed epithelial collectives in the presence or absence of RAB5A expression[138]. The velocity vectors (black arrows) of each individual centroid are shown. Jammed immobile, control monolayers display randomly oriented poorly motile cells. RAB5A expression increases cell velocity and promotes the alignment of the velocity vectors. This results in monolayers displaying a flocking mode of motion where long-range collective motion and short-range, local cell arrangements are permitted (not shown). The colour code indicates regions within the monolayer with velocity vectors that are either parallel (yellow) or antiparallel (blue) to the mean direction of migration.

Box 1. Clathrin-coated plaques: role in mechanosensing, adhesion and mitosis.
Clathrin-coated plaques (CPLs) are long-lived plasma membrane structures enriched in integrins and various signalling receptors but not actin[32,33], that are regulated by the physical properties of the extracellular matrix (ECM). As the substrate rigidity increases, CPLs assemble and expand. By linking CPLs to the substrate, integrin $\alpha v\beta 5$ delays their budding, in a process called "frustrated endocytosis", ultimately stabilizing

them[33]. Thus, CPLs might represent mechanosensitive adhesion platforms, generated as a consequence of "frustrated endocytosis"[33] (see figure). CPLs are present in interphase cells (left in figure), together with "canonical" focal adhesion complexes, which are enriched in integrins, focal adhesion kinase (FAK), actin, vinculin, talin and paxillin. At mitosis (right in figure), the actin cytoskeleton is reorganized to form a contractile cortical network of actin filaments, which promotes a cellular morphological change, known as "rounding up". During cell rounding for mitosis, a decrease in cell surface area is achieved and endocytosis continues, albeit at a lower rate, while recycling is impaired[288-290]. At the same time, "canonical" focal adhesion complexes are disassembled, while "mitotic" adhesion complexes are established to preserve the interaction with the substrate needed to achieve daughter cell re-spreading and mitotic spindle orientation[291-294]. These mitotic adhesion complexes and CPLs are likely the same type of structure[34] since they share several properties. Both types of structures are very stable with slow plasma membrane turnover; they are composed mainly of integrins, while actin is not enriched and does not play any role in their dynamics; they are enriched in endocytic proteins. In addition, their number and size increase with the increase of the rigidity of the substrate[293]. Another function of CPLs (not shown in the figure) is to act as adhesive structures in non-mitotic cells migrating in 3D environments. In this case, they are assembled on linear collagen fibres and provide multiple anchoring sites to the ECM along cell protrusions in order to support 3D cell migration, in cooperation with classical focal adhesions[295].

Glossary

Receptor tyrosine kinases
A family of plasma membrane proteins (~ 60 genes in humans) that function as high affinity binding sites for growth factors and cytokines and transduce signals intracellularly through their intrinsic tyrosine kinase activity.

G protein-coupled receptors
A vast family of plasma membrane receptors (more than 800 genes in humans) characterized by seven transmembrane regions. They transduce signals through a variety of modes, among which the best characterized one is the coupling with heterotrimeric G proteins.

Arrestins
A family of proteins that act as multifunctional scaffolding proteins, regulating trafficking and signaling of transmembrane receptors, particularly of GPCRs. They are involved in receptor desensitization, endocytosis and ubiquitylation. They can also function as positive effectors of GPCRs through their scaffolding abilities. The arrestin family comprises visual arrestins, β-arrestins (non-visual arrestins) and α-arrestins

Epsin family of endocytic adaptor proteins
A family of endocytic proteins composed of 3 paralogs: EPN1, EPN2 and EPN3, characterized by the presence of an epsin N-terminal homology (ENTH) domain involved in phosphoinositide binding at the plasma membrane, ubiquitin binding

motifs (UIMs), as well as motifs that bind to clathrin, AP2 and other endocytic proteins. They are involved in both CME, where they play a role in clathrin-coat assembly and cargo recruitment, and in EGFR-NCE.

Epithelial-mesenchymal transition (EMT)

This is a process, of great relevance in embryogenesis, through which epithelial cells lose polarity and cell–cell adhesion contacts (sessile state) to acquire characteristics of migratory mesenchymal-like cells. In physiology, typically, after migrating, these cells re-acquire an epithelial phenotype through the opposite process of mesenchymal--epithelial transition (MET).

Death receptors

Death receptors are type I transmembrane proteins belonging to the tumor necrosis factor/nerve growth factor superfamily. They are activated upon binding to various agonists (such as FASLG, TNFA or TRAIL). They typically trigger the so-called apoptotic extrinsic pathway, yet they can also activate multiple alternative signaling pathways with opposing outcomes (survival/proliferation *vs.* cell death) depending on the cell context.

Caveolae

Small flask-shaped invaginations of the plasma membrane (50–80 nm) that can be morphologically identified by the presence of coat-like proteins, caveolins, and that are particularly abundant in tissues involved in lipid homeostasis or subjected to mechanical challenges like adipocytes, muscle and endothelial cells.

Vinculin

A protein involved in the formation of focal adhesions that links surface structures (integrins) to the actin cytoskeleton (through binding to F-actin).

Focal adhesions

Cell-to-matrix adhesion structures involved in the transmission and regulation of signals between the extracellular matrix and the intracellular environment. They are large and dynamic protein complexes established through integrins (which bind to the extracellular matrix), vinculin, F-actin and several regulatory components (up to hundred different proteins, according to the state of the cell). Focal adhesions have roles in signal transduction, cell motility, cell cycle regulation and several other cellular phenotypes. They represent one of the main sensors/effectors in cellular mechanosensing.

Tip cells

During angiogenesis, new vessels that sprout from existing ones are guided by a leader cell that drives the extension of the sprout and senses the environment for guidance cues.

ERK signalling

Is the signalling mediated by the activation of the extracellular signal regulated kinases (ERKs, also called mitogen-activated protein kinases or MAPKs). This signalling is

mediated by the sequential activation of the small GTPases RAS, and a cascade of kinases (RAF, MEK, and ERK1,2) that transduce a signal from a receptor, located on the cell surface or on endosomes, to regulate as number of fundamental biological function, including cell proliferation, differentiation and migration.

PAK

A family of serine/threonine protein kinases that includes six members in mammals. They serve as targets for the small GTPases CDC42 and RAC and have been implicated in a wide range of biological activities.

PTEN

A lipid phosphatase (phosphatidylinositol 3,4,5 triphosphate 3-phosphatase). It catalyses the conversion of PI(3,4,5)P3 to PI(4,5)P2, thereby antagonizing the action of PI3K and the activation of AKT. It represents one of the most frequently lost tumor suppressors in human cancers

AMPK

AMP-activated protein kinase or 5' adenosine monophosphate-activated protein kinase. It is a heterotrimeric protein complex endowed with serine/threonine kinase activity which regulates the energy metabolism, mostly acting on glucose and fatty acid metabolism.

RAC1

A member of RHO subfamily of small GTPases that plays a central role in controlling the activity of protein complexes that are necessary to remodel the actin cytoskeleton during migration.

P38

A member of a class of mitogen-activated protein kinases (MAPKs) that are responsive to stress stimuli, such as cytokines, ultraviolet irradiation, heat shock, and osmotic shock, and are involved in cell differentiation, apoptosis and autophagy.

JNK

JNK (or c-Jun N-terminal Kinase), is a member of a family of protein kinases, which play a central role in stress signalling pathways implicated in gene expression, neuronal plasticity, regeneration, cell death, and regulation of cellular senescence.

RAB GTPases

A subfamily of small GTPases that includes more than 70 members in mammals and regulates several key steps of membrane trafficking, including vesicle formation, vesicle movement along actin and tubulin networks, and membrane fusion.

GEF

This term defines broadly a vast group of proteins (frequently unrelated) which all possess Guanine Nucleotide Exchange Factor activity, *i.e.*, the ability to convert G proteins from an inactive GDP-bound to an active GTP-bound form.

“Jammed” epithelial monolayers

The dynamics of epithelia has been described in terms of jamming transitions. During this transition, collective motion ceases, cells can no longer exchange neighbours, and monolayers become static and rigid, displaying a behaviour similar to that of ensembles of dense and packed inactive particles, such us coffee in a chute or sand in a pile.

WAVE

A key component of a pentameric actin nucleation promoting complex which acts downstream of the GTPase RAC, and is necessary for activating the ARP2/3 complex for the generation of branched actin networks.

Midbody

Central region of the thin cytoplasmic bridge that connects cells at the end of cytokinesis. It consists mostly of microtubules, together with various other types of proteins (400-500). It functions as a platform to mediate abscission, the process of severing the intercellular bridge. It is also endowed with numerous other functions, including determination of cell fate and asymmetric post-abscission signal transduction.

Tight junction

A cell-to-cell junction formed by a multiprotein complex. This type of junction is established through homotypic interactions between adhesion molecules (occludins, claudins, JAMs) present on the surface of abutting cells. Tight junctions mark the border between the apical and the basolateral surfaces in epithelial cells and control the formation of functionally distinct apical domains. They are also present in endothelial cells and astrocytes and establish the blood-brain barrier. One of their major function is to seal the epithelia by preventing leakage of water and small molecular weight solutes.

Arp2/3 complex

A seven-subunit complex that upon activation promotes the branched elongation of the actin network by binding to the side of mother filaments.

Crumbs polarity complex

A multiprotein complex composed of three members originally identified in *Drosophila*, Crumbs, Pals1 and PatJ. This complex plays a key role in specifying the apical plasma membrane domain of epithelial cells and in controlling cell shape in both invertebrates and vertebrates.

Transcytosis

A process in which molecules are transported across cellular barriers. It involves the endocytosis of molecules (typically plasma membrane proteins or extracellular molecules captured through interaction with surface receptors) at one side of the cell and their vesicle-mediated transport to another side, where they are released through exocytosis. It contributes to the establishment of apical-basal cell polarity by transferring transmembrane proteins between distinct plasma membrane domains. It

is involved in many other processes, for instance the crossing of the blood-brain barrier.

Exocyst
An octameric protein complex involved in vesicle trafficking, specifically in the tethering and spatial targeting of vesicles to the plasma membrane prior to vesicle fusion.

Annexin-2
A 36-kDa calcium-dependent, phospholipid-binding protein that functions in promoting the exocytosis of intracellular proteins to the extracellular space.

CDC42 apical polarity complex
CDC42 is a highly conserved RHO-family GTPase that regulates cell polarity in many eukaryotes. It directly interacts with PAR6, and regulates, through this protein, the activity of the atypical protein kinase C, aPKC.

BAR domain
This is a banana-shaped protein domain capable of sensing membrane curvature by binding preferentially to curved membranes. BAR domains are named after three proteins in which they were originally identified: BIN1, bridging interactor 1; AMPH, amphiphysin; and Rvs167, the yeast homolog of amphiphysin.

Adherens junctions
Cadherin-based cell-to-cell junctions present in epithelial and endothelial cells, frequently in a more basal position with respect to tight junctions.

Isochoric
A process in which the volume of a closed system does not change. It is synonym of isovolumetric.

Germband
In *Drosophila melanogaster*, the ventral part of the embryo that forms during gastrulation and gives rise to the segmented trunk of the animal (gnathal, thoracic, abdominal segments). It includes the mesoderm, ventral ectoderm and dorsal epidermis but excludes the dorsal-most tissue of the embryo, the amnioserosa.

Amnioserosa
In *Drosophila melanogaster*, a short-lived extraembryonic tissue with a critical role in dorsal closure and other early developmental morphogenetic events.

Border cells
A cluster of cells that migrate from the anterior tip of the Drosophila egg chamber to the border of the oocyte at stage 9 of Drosophila oogenesis. These cells perform a stereotypical collective migration on the intervening nurse cells, and reach the oocyte. They are required for the formation of the micropyle, the eggshell structure through which sperm enters the egg.

Lamellipodia
Thin membrane protrusion present at the leading edge of migrating cells, mostly constituted by a flat network of actin.

Treadmilling
A process characterizing filamentous multimeric protein structures within the cell and mostly used in reference to filamentous actin (F-actin). When actin subunits (G-actin) are constantly added at one end of the filament and removed from the opposite one, the net effect is the treadmilling of the filament which is used, for instance, to generate motion. The term is also used, more generally, for other biological processes in which "treadmilling" of molecules or organelles occurs.

Neural crest
A temporary group of cells established during vertebrate development, which forms after gastrulation at the border between the neural plate and the surrounding ectoderm. After closure of the neural tube (due to the folding of the neural plate into itself), the neural crest runs along the roof plate of the neural tube. At this stage, neural crest cells undergo epithelial-mesenchymal transition and migrate to the periphery, where they give origin to various cell lineages.

Ascitic fluid
An abnormal accumulation of fluid in the abdominal cavity frequently caused by liver disease or cirrhosis, cancers, specifically ovarian and colon cancer, and heart failure.

AKT kinase
The three members of the human AKT serine-threonine protein kinase family are often referred to as protein kinase B alpha, beta, and gamma. These proteins are phosphorylated by phosphoinositide 3-kinase (PI3K). AKT/PI3K forms a key component of many signalling pathways that involve the binding of membrane-bound ligands such as receptor tyrosine kinases.

Galectins
A class of proteins that bind specifically to β-galactoside sugars, such as N-acetyl-lactosamine. Galectins are secreted in the extracellular space, where they encounter galactose-containing glycoproteins and glycolipids. Binding of galectins to glycosylated proteins, such as CD44 and α5β1 integrin, triggers galectin oligomerisation, which allows their interaction with glycosphingolipids and the generation of plasma membrane curvature, leading to the formation of clathrin-independent endocytic carriers (CLICs).

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