AperTO - Archivio Istituzionale Open Access dell'Università di Torino

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

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1795208> since 2021-07-28T12:30:12Z

Published version:

DOI:10.1038/s41580-021-00375-5

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

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

Sara Sigismund^{1,2}, Letizia Lanzetti^{3,4}, Giorgio Scita^{2,5}, Pier Paolo Di Fiore^{1,2*}

¹ IEO, European Institute of Oncology IRCCS, Milan, Italy

² Department of Oncology and Haemato-Oncology, Università degli Studi di Milano, Milan Italy

³ Department of Oncology, University of Torino Medical School, Italy

⁴ Candiolo Cancer Institute, FPO - IRCCS, Candiolo, Torino, Italy

⁵ IFOM, the FIRC Institute of Molecular Oncology, Milan, Italy

* Corresponding author pierpaolo.difiore@ieo.it

Author contributions

The authors contributed equally to all aspects of the article.

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 space⁵. 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 recycling⁶⁻⁹.

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 behaviour¹⁰⁻¹³.

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 sites¹⁹⁻²¹, on endocytosis in neuronal communication and synaptic function²²⁻²⁴, and on the trafficking of adhesion molecules, such as integrins and cadherins²⁵⁻²⁷, 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 plaques²⁸⁻³³, which act as signalling and adhesion platforms³⁴, 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 dynamics⁴⁰ 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 signalling²⁸.

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 functions⁴². 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 $\beta 1$ -AR is endocytosis-incompetent, meaning it is not internalized, yet, is still able to induce β -arrestin accumulation and signalling in clathrin-coated pits⁴⁵⁻⁴⁷. 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 AP2⁴⁷.

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 proteins⁵²⁻⁵⁴. 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 cycles⁶². By contrast, dynamin2 (DYN2) is constitutively active and responsible for ubiquitous and constitutive endocytosis in all cell types⁶²⁻⁶⁶. 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 GSK3 β -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 cancer⁶⁹. DYN2 overexpression promotes invasiveness in different cancers, including hepatocellular carcinoma, prostate and pancreatic cancer⁷⁰⁻⁷². DYN1 is aberrantly activated in non-small cell lung cancer where GSK3 β 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 survival⁷⁴.

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 migration⁸⁰ and in the regulation of membrane tension in adherent cells⁸¹. 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 growth⁸³. 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/GEEC⁸⁵, 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 Ca²⁺ release from the ER, required for NCE vesicle fission⁸⁷. 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 pathway⁹⁴⁻⁹⁶. These RTKs are also internalized via FEME⁸⁴. 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 cells⁹⁸⁻¹⁰⁰.

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 regulation¹⁰². In macrophages and immature dendritic cells, macropinocytosis is constitutively active and critical for antigen presentation¹⁰². 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 cancer¹⁰² by activation of oncogenes, such as RAS and SRC¹⁰³⁻¹⁰⁵. Under nutrient-deprived conditions, when the supply from the vasculature is insufficient, cancer cells can scavenge nutrients (mainly albumin) from the extracellular environment by macropinocytosis¹⁰⁶. 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 formation¹⁰⁷. Context is again at play, since cancer cell lines displaying similar oncogenic alterations vary in the extent and modality (constitutive or inducible) of necrocytosis¹⁰⁸. Importantly, necrocytosis promotes therapy resistance as it relieves stress induced by drugs targeting anabolic pathways¹⁰⁸; thus, its inhibition could be exploited to target therapy resistant tumours¹⁰⁹.

[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 JNK¹¹¹. 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 nucleus¹¹⁷, 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 signalling¹¹⁸⁻¹²¹. 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 output¹²²⁻¹²⁴. This has enormous therapeutic implications. GPCRs are easily druggable: approximately 35% of all approved drugs are directed against them¹²⁵. 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 output¹²⁶. 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 antagonists¹²⁷⁻¹²⁹. These types of drugs may also benefit cancer patients; this is a particularly urgent need since, despite extensive knowledge of altered GPCR signalling¹³⁰⁻¹³² in tumours, only a few anti-GPCR drugs are approved for therapy in this disease¹³³.

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 healing¹³⁶ and their propagation serves as a directional cue for collective cell migration¹³⁷. 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 differentiation¹³⁹.

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 patterning¹⁴⁴⁻¹⁴⁹. 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 expression¹⁵⁰), signalling pathways that they impinge upon, and possibly subcellular localization¹⁵¹⁻¹⁵³. 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-RAS¹⁵⁶. Such non-linear dynamics of recruitment and activation are typical of self-organizing systems that form spatial patterns on membranes¹⁵⁷, 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 pathway¹⁵⁸.

[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 transformation¹⁶³.

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 tissues¹⁶⁰⁻¹⁶². 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 cells¹⁶⁴. 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 GTPases¹⁶⁵, 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 polarity¹⁶⁶. 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-dependency¹⁶⁶.

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 structures¹⁶⁷ (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 available¹⁶⁸⁻¹⁷⁰.

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 proteins¹⁷². 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 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 site¹⁷⁶, 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 PAR6¹⁷⁸. 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 lumens¹⁷⁹.

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 space¹⁸².

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 proteins¹⁸³. A number of polarity determinants – including PARD6B, SCRIB, PRKCI and DLGs – are amplified or display aberrant cellular distribution in cancer¹⁸³. 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 biology¹⁶³. 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 systems¹⁹⁰, 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 neighbours¹⁹³. 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 cells¹⁹⁴. Indeed, during germband extension in *D. melanogaster*, clathrin and AP2 become enriched at the contractile interface of neighbouring cells in the antero-posterior direction¹⁹⁵; here, polarized actomyosin contractility drives clustering and internalization of E-Cadherin, which leads to junctional shrinkage by reducing local cell–cell adhesion¹⁹⁵.

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 tissues¹⁹⁷. In the former example (Figure 5b), adherens junctions tune their length and maintain junctional straightness and tension while the whole tissue undergoes a global contraction¹⁹⁸.

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 endocytosis¹⁹⁸. 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 contractility¹⁹⁷. 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 endocytosis¹⁹⁷.

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 cells²⁰¹ and integrin trafficking during glandular morphogenesis¹⁶⁶, 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 macropinocytosis²⁰² 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 kinase²⁰³. 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 perturbed²⁰⁴.

[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 environment²¹⁴. 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 dynamics²¹⁵. 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 wounding²¹⁶. 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 junctions²¹⁶, 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-Cadherin²¹⁹. 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 rearrangement²¹⁹. 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

breakdown LPA, generating outward-facing local gradients — low in the tumour and high in the surrounding environment — that guide metastatic cell dispersal through a self-generated LPA chemotactic gradient^{220,221}. Engagement of the LPAR stimulates its endocytosis in early endosomes. N-WASP, a promoter of branched actin polymerization, together with the endocytic protein SNX18, coordinate recycling of internalized LPAR from RAB11–RAB8-double positive endocytic tubules back to the plasma membrane preventing its degradation. The N-WASP-mediated LPAR recycling then ensures constant replenishment of the receptor at the plasma membrane allowing persistent RHOA activation, resulting in increased cell contractility, generation of traction forces and matrix remodelling for efficient cell migration. Loss of N-WASP affects invasiveness of pancreatic cancer cells pointing to N-WASP-dependent chemotaxis to LPA gradients as a motivator of egress from primary tumours and tropism towards metastatic sites²²¹. In HeLa cells, the hyperactivation of the LPAR might also occur through trapping the receptor at the plasma membrane in coated plaques²²² (Box 1): reduced LPAR internalization prolongs its downstream signalling, representing an alternative mechanism to recycling for regulating actomyosin-based contractility. Whether coated plaques are operative in pancreatic cancer cells remains an issue for future investigations; notably, these structures are increased in cells plated on stiff substrates³³, suggesting that the hyper-stiff, stromal-rich, microenvironment of pancreatic cancer cells might favour their formation.

These findings highlight how an integrated biochemical and physical perspective is necessary for a holistic comprehension of the dynamics of multicellular entities and tissues. Such dynamics can be better understood by considering cell collectives as ensembles of “active particulate matter” that are, nevertheless, governed by structural and dynamic physical properties typical of amorphous viscoelastic materials^{223,224}. During tissue growth, cells are free to move, as in a fluid, but their motion becomes constrained as cell density increases. At a critical cell density, motility ceases and collectives rigidify undergoing a liquid-like to solid-like transition referred to as tissue jamming²²⁵⁻²²⁹. This transition is thought to promote the development of elasticity and of barrier properties, but also to act as a suppressive mechanism for the growth of oncogenic clones.

Recently, it was shown that global perturbations of endocytosis — achieved by modulating the master regulator of early endosome biogenesis, RAB5A — impinges on biomechanical properties of cellular collectives. Increased RAB5A levels were sufficient to re-awaken the motility of “jammed” epithelial monolayers by triggering millimetre-scale, coherent and ballistic locomotion of multicellular streams that flow like “flocking” fluids²³⁰, through the modulation of EGFR signalling (Figure 6b and 6c). In mammary epithelial cells, RAB5A induced EGFR-NCE, leading to accumulation of activated receptors in endosomes, whose size and number were remarkably elevated²³¹, and to elevation of endosomal ERK1/2 signalling, in keeping with the concept that endosomes act as quanta-like platforms where phosphorylated EGFR can be packaged at constant mean amounts¹³⁹. In turn, this caused the hyper-phosphorylation of the Arp2/3 nucleating promoting factor, WAVE2, fuelling the extension of lamellipodia and directed cell motility²³¹ (Figure 6b).

Tumour cells can exploit this endocytic-mediated regulation of cell mechanics to facilitate their collective dissemination. Consistently, mammary tumour spheroids overexpressing RAB5 display persistent angular rotation and elevated local cell

rearrangements²³¹. 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 models²³². 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 axis²³³. 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 properties²³⁴. 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 state²³⁵. Physiologically, it is involved in morphogenetic events, including gastrulation and neural crest migration²³⁶. Pathologically, EMT is exploited by cancer cells to acquire invasive/metastatic ability²³⁷⁻²³⁹ — a process also connected with the acquisition of a cancer stem cell-like phenotype and drug resistance²⁴⁰⁻²⁴³.

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 ZEB1/2²⁴⁶⁻²⁴⁸. 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 niche²⁴⁹. 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 factors²⁵⁰. 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 EMT²⁵⁰. Furthermore, it has been shown that cancer cells adopting different degrees of EMT, tend to metastasize to different sites²⁵¹ 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 signalling²⁵²⁻²⁵⁴, 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 coats⁴⁰. These differences hinged on context-dependent variations in AP2 levels and in the requirement for PI3K activity⁴⁰. Another approach is to employ organoids that recapitulate the complex differentiation patterns that occur in vivo²⁵⁶. 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.

Acknowledgments

We thank Maria Grazia Malabarba for support in figure design and Rosalind Gunby for critically editing the manuscript before submission. Work in the authors’ labs is supported by: Associazione Italiana per la Ricerca sul Cancro (AIRC IG 24415 to SS, AIRC IG 22811 to LL, AIRC IG 18621 and 5XMille 22759 to GS, and AIRC IG 18988, AIRC IG 23060 and MCO 10000 to PPDF); the Worldwide Cancer Research (20-0094 to SS), the Italian Ministry of University and Scientific Research (PRIN 2017, Prot. 2017E5L5P3 to SS; Prot. 2017HWTP2K to GS; PRIN 2015 Prot. 2015XS92CC to PPDF); the University of Milan (PSR2019 to SS); the FPRC 5x1000 Ministero Salute 2017 (to LL); the Italian Ministry of Health (RF-2016-02361540 to PPDF).

We apologize to the many colleagues whose excellent work could not be reviewed or cited for lack of space. An additional number of primary research papers and reviews is included in Supplementary Information.

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 only⁴⁸⁻⁵¹. After endocytosis, the iron bound to Tf is released in an endosomal compartment and the Tf–TfR complex is recycled back to the surface²⁵⁷. **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 surface²⁶⁴. **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 membrane¹¹¹. 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” signalling⁴¹⁻⁴⁴. **d.** Epidermal growth factor receptor (EGFR) can be internalized through different CCPs⁵¹: 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 degradation⁵¹. **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 GSK3 β -dependent DYN1 phosphorylation are removed^{36,65,68}. In cancer, the GSK3 β 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/GEEC⁸⁵, 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 Ca²⁺ 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 CME²⁶⁷. 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 occur²⁶⁸. 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–STAT²⁷⁰. 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 degraded⁹¹. **e.** Macropinocytosis internalizes pathogens, apoptotic cells/debris and proteins through the formation of actin-dependent plasma membrane protrusions¹⁰¹. 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 presentation¹⁰². 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 shown²⁷⁴. 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 fates²⁷⁶. 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 surface²⁷⁶. 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 cells²⁸⁰. 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 extension¹⁹³ (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 tissues¹⁹⁷. 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 interfaces¹⁹⁶ (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 expression¹³⁸. 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 $\alpha\text{v}\beta 5$ delays their budding, in a process called “frustrated endocytosis”, ultimately stabilizing

them³³. 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 impaired²⁸⁸⁻²⁹⁰. 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 orientation²⁹¹⁻²⁹⁴. These mitotic adhesion complexes and CPLs are likely the same type of structure³⁴ 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 substrate²⁹³. 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 adhesions²⁹⁵.

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 a 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 as 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 $\alpha 5\beta 1$ 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).

References

- 1 Sigismund, S. *et al.* Endocytosis and signaling: cell logistics shape the eukaryotic cell plan. *Physiol Rev* **92**, 273-366, doi:10.1152/physrev.00005.2011 (2012).

1268 2 Thottacherry, J. J., Sathe, M., Prabhakara, C. & Mayor, S. Spoiled for Choice:
1269 Diverse Endocytic Pathways Function at the Cell Surface. *Annu Rev Cell Dev Biol*
1270 **35**, 55-84, doi:10.1146/annurev-cellbio-100617-062710 (2019).

1271 3 Goh, L. K. & Sorkin, A. Endocytosis of receptor tyrosine kinases. *Cold Spring Harb*
1272 *Perspect Biol* **5**, a017459, doi:10.1101/cshperspect.a017459 (2013).

1273 4 Irannejad, R., Tsvetanova, N. G., Lobingier, B. T. & von Zastrow, M. Effects of
1274 endocytosis on receptor-mediated signaling. *Curr Opin Cell Biol* **35**, 137-143,
1275 doi:10.1016/j.ceb.2015.05.005 (2015).

1276 5 Villaseñor, R., Kalaidzidis, Y. & Zerial, M. Signal processing by the endosomal
1277 system. *Curr Opin Cell Biol* **39**, 53-60, doi:10.1016/j.ceb.2016.02.002 (2016).

1278 6 Lanzetti, L. & Di Fiore, P. P. Behind the Scenes: Endo/Exocytosis in the Acquisition
1279 of Metastatic Traits. *Cancer Res* **77**, 1813-1817, doi:10.1158/0008-5472.CAN-16-
1280 3403 (2017).

1281 7 Wilson, B. J., Allen, J. L. & Caswell, P. T. Vesicle trafficking pathways that direct
1282 cell migration in 3D matrices and in vivo. *Traffic* **19**, 899-909,
1283 doi:10.1111/tra.12605 (2018).

1284 8 Disanza, A., Frittoli, E., Palamidessi, A. & Scita, G. Endocytosis and spatial
1285 restriction of cell signaling. *Mol Oncol* **3**, 280-296,
1286 doi:10.1016/j.molonc.2009.05.008 (2009).

1287 9 Eaton, S. & Martin-Belmonte, F. Cargo sorting in the endocytic pathway: a key
1288 regulator of cell polarity and tissue dynamics. *Cold Spring Harb Perspect Biol* **6**,
1289 a016899, doi:10.1101/cshperspect.a016899 (2014).

1290 10 Sigismund, S. & Scita, G. The 'endocytic matrix reloaded' and its impact on the
1291 plasticity of migratory strategies. *Curr Opin Cell Biol* **54**, 9-17,
1292 doi:10.1016/j.ceb.2018.02.006 (2018).

1293 11 Katsuno-Kambe, H. & Yap, A. S. Endocytosis, cadherins and tissue dynamics.
1294 *Traffic* **21**, 268-273, doi:10.1111/tra.12721 (2020).

1295 12 Ladoux, B., Mege, R. M. & Treppe, X. Front-Rear Polarization by Mechanical Cues:
1296 From Single Cells to Tissues. *Trends Cell Biol* **26**, 420-433,
1297 doi:10.1016/j.tcb.2016.02.002 (2016).

1298 13 Gonzalez-Gaitan, M. & Julicher, F. The role of endocytosis during morphogenetic
1299 signaling. *Cold Spring Harb Perspect Biol* **6**, a016881,
1300 doi:10.1101/cshperspect.a016881 (2014).

1301 14 Mettlen, M. *et al.* Endocytic accessory proteins are functionally distinguished by
1302 their differential effects on the maturation of clathrin-coated pits. *Mol Biol Cell*
1303 **20**, 3251-3260, doi:10.1091/mbc.E09-03-0256 (2009).

1304 15 Johannes, L., Parton, R. G., Bassereau, P. & Mayor, S. Building endocytic pits
1305 without clathrin. *Nat Rev Mol Cell Biol* **16**, 311-321, doi:10.1038/nrm3968 (2015).

1306 16 Ferreira, A. P. A. & Boucrot, E. Mechanisms of Carrier Formation during Clathrin-
1307 Independent Endocytosis. *Trends Cell Biol* **28**, 188-200,
1308 doi:10.1016/j.tcb.2017.11.004 (2018).

1309 17 Azarnia Tehran, D., Lopez-Hernandez, T. & Maritzen, T. Endocytic Adaptor
1310 Proteins in Health and Disease: Lessons from Model Organisms and Human
1311 Mutations. *Cells* **8**, doi:10.3390/cells8111345 (2019).

1312 18 Khan, I. & Steeg, P. S. Endocytosis: a pivotal pathway for regulating metastasis. *Br*
1313 *J Cancer*, doi:10.1038/s41416-020-01179-8 (2020).

1314 19 Martello, A., Platt, F. M. & Eden, E. R. Staying in touch with the endocytic
1315 network: The importance of contacts for cholesterol transport. *Traffic* **21**, 354-
1316 363, doi:10.1111/tra.12726 (2020).

1317 20 Wu, H., Carvalho, P. & Voeltz, G. K. Here, there, and everywhere: The importance
1318 of ER membrane contact sites. *Science* **361**, doi:10.1126/science.aan5835 (2018).

1319 21 Scorrano, L. *et al.* Coming together to define membrane contact sites. *Nat*
1320 *Commun* **10**, 1287, doi:10.1038/s41467-019-09253-3 (2019).

1321 22 Kaempfer, N. & Maritzen, T. Safeguards of Neurotransmission: Endocytic Adaptors
1322 as Regulators of Synaptic Vesicle Composition and Function. *Front Cell Neurosci*
1323 **11**, 320, doi:10.3389/fncel.2017.00320 (2017).

1324 23 Saheki, Y. & De Camilli, P. Synaptic vesicle endocytosis. *Cold Spring Harb Perspect*
1325 *Biol* **4**, a005645, doi:10.1101/cshperspect.a005645 (2012).

1326 24 Soykan, T., Maritzen, T. & Haucke, V. Modes and mechanisms of synaptic vesicle
1327 recycling. *Curr Opin Neurobiol* **39**, 17-23, doi:10.1016/j.conb.2016.03.005 (2016).

1328 25 Moreno-Layseca, P., Icha, J., Hamidi, H. & Ivaska, J. Integrin trafficking in cells
1329 and tissues. *Nat Cell Biol* **21**, 122-132, doi:10.1038/s41556-018-0223-z (2019).

1330 26 Bruser, L. & Bogdan, S. Adherens Junctions on the Move-Membrane Trafficking
1331 of E-Cadherin. *Cold Spring Harb Perspect Biol* **9**,
1332 doi:10.1101/cshperspect.a029140 (2017).

1333 27 Nino, C. A., Sala, S. & Polo, S. When ubiquitin meets E-cadherin: Plasticity of the
1334 epithelial cellular barrier. *Semin Cell Dev Biol* **93**, 136-144,
1335 doi:10.1016/j.semcdb.2018.12.005 (2019).

1336 28 Lampe, M., Vassilopoulos, S. & Merrifield, C. Clathrin coated pits, plaques and
1337 adhesion. *J Struct Biol* **196**, 48-56, doi:10.1016/j.jsb.2016.07.009 (2016).

1338 29 De Deyne, P. G. *et al.* The vitronectin receptor associates with clathrin-coated
1339 membrane domains via the cytoplasmic domain of its beta5 subunit. *J Cell Sci*
1340 **111 (Pt 18)**, 2729-2740 (1998).

1341 30 Heuser, J. Three-dimensional visualization of coated vesicle formation in
1342 fibroblasts. *J Cell Biol* **84**, 560-583, doi:10.1083/jcb.84.3.560 (1980).

1343 31 Maupin, P. & Pollard, T. D. Improved preservation and staining of HeLa cell actin
1344 filaments, clathrin-coated membranes, and other cytoplasmic structures by
1345 tannic acid-glutaraldehyde-saponin fixation. *J Cell Biol* **96**, 51-62,
1346 doi:10.1083/jcb.96.1.51 (1983).

1347 32 Saffarian, S., Cocucci, E. & Kirchhausen, T. Distinct dynamics of endocytic
1348 clathrin-coated pits and coated plaques. *PLoS Biol* **7**, e1000191,
1349 doi:10.1371/journal.pbio.1000191 (2009).

1350 33 Baschieri, F. *et al.* Frustrated endocytosis controls contractility-independent
1351 mechanotransduction at clathrin-coated structures. *Nat Commun* **9**, 3825,
1352 doi:10.1038/s41467-018-06367-y (2018).

1353 **This paper describes a function for clathrin-coated plaques as contractility-**
1354 **independent mechanosensitive structures that assemble with increasing**
1355 **substrate rigidity and that serve as platforms for receptor tyrosine kinase**
1356 **signaling.**

1357 34 Lock, J. G. *et al.* Clathrin-containing adhesion complexes. *J Cell Biol* **218**, 2086-
1358 2095, doi:10.1083/jcb.201811160 (2019).

- 35 Kirchhausen, T., Owen, D. & Harrison, S. C. Molecular structure, function, and dynamics of clathrin-mediated membrane traffic. *Cold Spring Harb Perspect Biol* **6**, a016725, doi:10.1101/cshperspect.a016725 (2014).
- 36 Mettlen, M., Chen, P. H., Srinivasan, S., Danuser, G. & Schmid, S. L. Regulation of Clathrin-Mediated Endocytosis. *Annu Rev Biochem* **87**, 871-896, doi:10.1146/annurev-biochem-062917-012644 (2018).
- 37 Antonny, B. *et al.* Membrane fission by dynamin: what we know and what we need to know. *EMBO J* **35**, 2270-2284, doi:10.15252/embj.201694613 (2016).
- 38 Ramachandran, R. & Schmid, S. L. The dynamin superfamily. *Curr Biol* **28**, R411-R416, doi:10.1016/j.cub.2017.12.013 (2018).
- 39 Kaksonen, M. & Roux, A. Mechanisms of clathrin-mediated endocytosis. *Nat Rev Mol Cell Biol* **19**, 313-326, doi:10.1038/nrm.2017.132 (2018).
- 40 Dambournet, D. *et al.* Genome-edited human stem cells expressing fluorescently labeled endocytic markers allow quantitative analysis of clathrin-mediated endocytosis during differentiation. *J Cell Biol* **217**, 3301-3311, doi:10.1083/jcb.201710084 (2018).
- Using genome-edited human embryonic stem cells to derive isogenic fibroblasts and neuronal progenitors, the authors show that the levels of expression of the endocytic adaptor AP2 are cell context-regulated and that this impinges on CME dynamics.**
- 41 Hanyaloglu, A. C. & von Zastrow, M. Regulation of GPCRs by endocytic membrane trafficking and its potential implications. *Annu Rev Pharmacol Toxicol* **48**, 537-568, doi:10.1146/annurev.pharmtox.48.113006.094830 (2008).
- 42 Puthenveedu, M. A. & von Zastrow, M. Cargo regulates clathrin-coated pit dynamics. *Cell* **127**, 113-124, doi:10.1016/j.cell.2006.08.035 (2006).
- 43 Henry, A. G. *et al.* Regulation of endocytic clathrin dynamics by cargo ubiquitination. *Dev Cell* **23**, 519-532, doi:10.1016/j.devcel.2012.08.003 (2012).
- 44 Soohoo, A. L. & Puthenveedu, M. A. Divergent modes for cargo-mediated control of clathrin-coated pit dynamics. *Mol Biol Cell* **24**, 1725-1734, S1721-1712, doi:10.1091/mbc.E12-07-0550 (2013).
- 45 Eichel, K., Jullie, D. & von Zastrow, M. beta-Arrestin drives MAP kinase signalling from clathrin-coated structures after GPCR dissociation. *Nat Cell Biol* **18**, 303-310, doi:10.1038/ncb3307 (2016).
- 46 Eichel, K. *et al.* Catalytic activation of beta-arrestin by GPCRs. *Nature* **557**, 381-386, doi:10.1038/s41586-018-0079-1 (2018).
- This study demonstrates an additional mechanism of β -arrestin activation, which does not require a stable GPCR- β -arrestin binding and promotes the accumulation of β -arrestin in clathrin-coated pits after dissociation from the GPCR, leading to ERK signalling.**
- 47 Latorraca, N. R. *et al.* Molecular mechanism of GPCR-mediated arrestin activation. *Nature* **557**, 452-456, doi:10.1038/s41586-018-0077-3 (2018).
- The mechanism of receptor-mediated arrestin activation is herein investigated through atomic-level simulations, revealing that, in the absence of a receptor, arrestin frequently adopts active conformations, which may explain why arrestin may remain active also after receptor dissociation (as shown by Eichel *et al.* (2018)).**

1405 48 Huang, F., Khvorova, A., Marshall, W. & Sorkin, A. Analysis of clathrin-mediated
1406 endocytosis of epidermal growth factor receptor by RNA interference. *J Biol*
1407 *Chem* **279**, 16657-16661, doi:10.1074/jbc.C400046200 (2004).

1408 49 Motley, A., Bright, N. A., Seaman, M. N. & Robinson, M. S. Clathrin-mediated
1409 endocytosis in AP-2-depleted cells. *J Cell Biol* **162**, 909-918,
1410 doi:10.1083/jcb.200305145 (2003).

1411 50 Hinrichsen, L., Harborth, J., Andrees, L., Weber, K. & Ungewickell, E. J. Effect of
1412 clathrin heavy chain- and alpha-adaptin-specific small inhibitory RNAs on
1413 endocytic accessory proteins and receptor trafficking in HeLa cells. *J Biol Chem*
1414 **278**, 45160-45170, doi:10.1074/jbc.M307290200 (2003).

1415 51 Pascolutti, R. *et al.* Molecularly Distinct Clathrin-Coated Pits Differentially Impact
1416 EGFR Fate and Signaling. *Cell Rep* **27**, 3049-3061 e3046,
1417 doi:10.1016/j.celrep.2019.05.017 (2019).

1418 52 Ko, G. *et al.* Selective high-level expression of epsin 3 in gastric parietal cells,
1419 where it is localized at endocytic sites of apical canaliculi. *Proc Natl Acad Sci U S*
1420 *A* **107**, 21511-21516, doi:10.1073/pnas.1016390107 (2010).

1421 53 Schiano Lomoriello, I. *et al.* A self-sustaining endocytic-based loop promotes
1422 breast cancer plasticity leading to aggressiveness and pro-metastatic behavior.
1423 *Nat Commun* **11**, 3020, doi:10.1038/s41467-020-16836-y (2020).

1424 **This study shows that the endocytic protein EPN3 is an oncogene with**
1425 **prognostic relevance in breast cancer and that it drives breast tumorigenesis**
1426 **through the induction of E-cadherin endocytosis, EMT and invasive behaviour.**

1427 54 Sen, A., Madhivanan, K., Mukherjee, D. & Aguilar, R. C. The epsin protein family:
1428 coordinators of endocytosis and signaling. *Biomol Concepts* **3**, 117-126,
1429 doi:10.1515/bmc-2011-0060 (2012).

1430 55 Kazacic, M. *et al.* Epsin 1 is involved in recruitment of ubiquitinated EGF
1431 receptors into clathrin-coated pits. *Traffic* **10**, 235-245, doi:10.1111/j.1600-
1432 0854.2008.00858.x (2009).

1433 56 Sigismund, S. *et al.* Clathrin-independent endocytosis of ubiquitinated cargos.
1434 *Proc Natl Acad Sci U S A* **102**, 2760-2765, doi:10.1073/pnas.0409817102 (2005).

1435 57 Chang, B. *et al.* Epsin is required for Dishevelled stability and Wnt signalling
1436 activation in colon cancer development. *Nat Commun* **6**, 6380,
1437 doi:10.1038/ncomms7380 (2015).

1438 58 Tian, X., Hansen, D., Schedl, T. & Skeath, J. B. Epsin potentiates Notch pathway
1439 activity in *Drosophila* and *C. elegans*. *Development* **131**, 5807-5815,
1440 doi:10.1242/dev.01459 (2004).

1441 59 Langridge, P. D. & Struhl, G. Epsin-Dependent Ligand Endocytosis Activates Notch
1442 by Force. *Cell* **171**, 1383-1396 e1312, doi:10.1016/j.cell.2017.10.048 (2017).

1443 60 Pasula, S. *et al.* Endothelial epsin deficiency decreases tumor growth by
1444 enhancing VEGF signaling. *J Clin Invest* **122**, 4424-4438, doi:10.1172/JCI64537
1445 (2012).

1446 61 Spradling, K. D., McDaniel, A. E., Lohi, J. & Pilcher, B. K. Epsin 3 is a novel
1447 extracellular matrix-induced transcript specific to wounded epithelia. *J Biol Chem*
1448 **276**, 29257-29267, doi:10.1074/jbc.M101663200 (2001).

1449 62 Ferguson, S. M. *et al.* A selective activity-dependent requirement for dynamin 1
1450 in synaptic vesicle endocytosis. *Science* **316**, 570-574,
1451 doi:10.1126/science.1140621 (2007).

- 1452 63 Ferguson, S. M. *et al.* Coordinated actions of actin and BAR proteins upstream of
1453 dynamin at endocytic clathrin-coated pits. *Dev Cell* **17**, 811-822,
1454 doi:10.1016/j.devcel.2009.11.005 (2009).
- 1455 64 Liu, Y. W., Surka, M. C., Schroeter, T., Lukiyanchuk, V. & Schmid, S. L. Isoform and
1456 splice-variant specific functions of dynamin-2 revealed by analysis of conditional
1457 knock-out cells. *Mol Biol Cell* **19**, 5347-5359, doi:10.1091/mbc.E08-08-0890
1458 (2008).
- 1459 65 Reis, C. R. *et al.* Crosstalk between Akt/GSK3beta signaling and dynamin-1
1460 regulates clathrin-mediated endocytosis. *EMBO J* **34**, 2132-2146,
1461 doi:10.15252/embj.201591518 (2015).
- 1462 66 Srinivasan, S. *et al.* A noncanonical role for dynamin-1 in regulating early stages
1463 of clathrin-mediated endocytosis in non-neuronal cells. *PLoS Biol* **16**, e2005377,
1464 doi:10.1371/journal.pbio.2005377 (2018).
- 1465 **Shows that although highly enriched in neurons, Dynamin-1 is expressed in**
1466 **non-neuronal cells, but inactivated via phosphorylation by GSK3 β , and that it**
1467 **becomes activated downstream of EGFR signalling to regulate dynamics of**
1468 **clathrin-coated pits.**
- 1469 67 Loerke, D. *et al.* Cargo and dynamin regulate clathrin-coated pit maturation. *PLoS*
1470 *Biol* **7**, e57, doi:10.1371/journal.pbio.1000057 (2009).
- 1471 68 Clayton, E. L. *et al.* Dynamin I phosphorylation by GSK3 controls activity-
1472 dependent bulk endocytosis of synaptic vesicles. *Nat Neurosci* **13**, 845-851,
1473 doi:10.1038/nn.2571 (2010).
- 1474 69 Meng, J. Distinct functions of dynamin isoforms in tumorigenesis and their
1475 potential as therapeutic targets in cancer. *Oncotarget* **8**, 41701-41716,
1476 doi:10.18632/oncotarget.16678 (2017).
- 1477 70 Gong, C. *et al.* Dynamin2 downregulation delays EGFR endocytic trafficking and
1478 promotes EGFR signaling and invasion in hepatocellular carcinoma. *Am J Cancer*
1479 *Res* **5**, 702-713 (2015).
- 1480 71 Ezratty, E. J., Bertaux, C., Marcantonio, E. E. & Gundersen, G. G. Clathrin
1481 mediates integrin endocytosis for focal adhesion disassembly in migrating cells. *J*
1482 *Cell Biol* **187**, 733-747, doi:10.1083/jcb.200904054 (2009).
- 1483 72 Burton, K. M. *et al.* Dynamin 2 interacts with alpha-actinin 4 to drive tumor cell
1484 invasion. *Mol Biol Cell* **31**, 439-451, doi:10.1091/mbc.E19-07-0395 (2020).
- 1485 73 Bhave, M., Mettlen, M., Wang, X. & Schmid, S. L. Early and non-redundant
1486 functions of dynamin isoforms in clathrin-mediated endocytosis. *Mol Biol Cell*,
1487 mbcE20060363, doi:10.1091/mbc.E20-06-0363 (2020).
- 1488 74 Reis, C. R., Chen, P. H., Bendris, N. & Schmid, S. L. TRAIL-death receptor
1489 endocytosis and apoptosis are selectively regulated by dynamin-1 activation.
1490 *Proc Natl Acad Sci U S A* **114**, 504-509, doi:10.1073/pnas.1615072114 (2017).
- 1491 75 Parton, R. G. *et al.* Caveolae: The FAQs. *Traffic*, doi:10.1111/tra.12689 (2019).
- 1492 76 Parton, R. G., McMahon, K. A. & Wu, Y. Caveolae: Formation, dynamics, and
1493 function. *Curr Opin Cell Biol* **65**, 8-16, doi:10.1016/j.ceb.2020.02.001 (2020).
- 1494 77 Sabharanjak, S., Sharma, P., Parton, R. G. & Mayor, S. GPI-anchored proteins are
1495 delivered to recycling endosomes via a distinct cdc42-regulated, clathrin-
1496 independent pinocytic pathway. *Dev Cell* **2**, 411-423, doi:10.1016/s1534-
1497 5807(02)00145-4 (2002).

1498 78 Kirkham, M. *et al.* Ultrastructural identification of uncoated caveolin-
1499 independent early endocytic vehicles. *J Cell Biol* **168**, 465-476,
1500 doi:10.1083/jcb.200407078 (2005).

1501 79 Holst, M. R. *et al.* Clathrin-Independent Endocytosis Suppresses Cancer Cell
1502 Blebbing and Invasion. *Cell Rep* **20**, 1893-1905, doi:10.1016/j.celrep.2017.08.006
1503 (2017).

1504 80 Howes, M. T. *et al.* Clathrin-independent carriers form a high capacity endocytic
1505 sorting system at the leading edge of migrating cells. *J Cell Biol* **190**, 675-691,
1506 doi:10.1083/jcb.201002119 (2010).

1507 81 Thottacherry, J. J. *et al.* Mechanochemical feedback control of dynamin
1508 independent endocytosis modulates membrane tension in adherent cells. *Nat*
1509 *Commun* **9**, 4217, doi:10.1038/s41467-018-06738-5 (2018).

1510 **This study describes a role for CLIC/GEEC endocytosis as a critical regulator of**
1511 **membrane tension in adherent cells, and dissects the downstream molecular**
1512 **mechanism, which involves vinculin as a mechanotransducer at focal adhesion**
1513 **sites.**

1514 82 Goldmann, W. H. Role of vinculin in cellular mechanotransduction. *Cell Biol Int*
1515 **40**, 241-256, doi:10.1002/cbin.10563 (2016).

1516 83 del Pozo, M. A. *et al.* Phospho-caveolin-1 mediates integrin-regulated membrane
1517 domain internalization. *Nat Cell Biol* **7**, 901-908, doi:10.1038/ncb1293 (2005).

1518 84 Boucrot, E. *et al.* Endophilin marks and controls a clathrin-independent endocytic
1519 pathway. *Nature* **517**, 460-465, doi:10.1038/nature14067 (2015).

1520 85 Casamento, A. & Boucrot, E. Molecular mechanism of Fast Endophilin-Mediated
1521 Endocytosis. *Biochem J* **477**, 2327-2345, doi:10.1042/BCJ20190342 (2020).

1522 86 Sigismund, S. *et al.* Clathrin-mediated internalization is essential for sustained
1523 EGFR signaling but dispensable for degradation. *Dev Cell* **15**, 209-219,
1524 doi:10.1016/j.devcel.2008.06.012 (2008).

1525 87 Caldieri, G. *et al.* Reticulon 3-dependent ER-PM contact sites control EGFR
1526 nonclathrin endocytosis. *Science* **356**, 617-624, doi:10.1126/science.aah6152
1527 (2017).

1528 88 Ghosh, S., Marrocco, I. & Yarden, Y. Roles for receptor tyrosine kinases in tumor
1529 progression and implications for cancer treatment. *Adv Cancer Res* **147**, 1-57,
1530 doi:10.1016/bs.acr.2020.04.002 (2020).

1531 89 Schlessinger, J. Receptor tyrosine kinases: legacy of the first two decades. *Cold*
1532 *Spring Harb Perspect Biol* **6**, doi:10.1101/cshperspect.a008912 (2014).

1533 90 Lamaze, C. *et al.* Interleukin 2 receptors and detergent-resistant membrane
1534 domains define a clathrin-independent endocytic pathway. *Mol Cell* **7**, 661-671,
1535 doi:10.1016/s1097-2765(01)00212-x (2001).

1536 91 Hemar, A. *et al.* Endocytosis of interleukin 2 receptors in human T lymphocytes:
1537 distinct intracellular localization and fate of the receptor alpha, beta, and gamma
1538 chains. *J Cell Biol* **129**, 55-64, doi:10.1083/jcb.129.1.55 (1995).

1539 92 Sehat, B., Andersson, S., Girnita, L. & Larsson, O. Identification of c-Cbl as a new
1540 ligase for insulin-like growth factor-I receptor with distinct roles from Mdm2 in
1541 receptor ubiquitination and endocytosis. *Cancer Res* **68**, 5669-5677,
1542 doi:10.1158/0008-5472.CAN-07-6364 (2008).

1543 93 Salani, B. *et al.* IGF-IR internalizes with Caveolin-1 and PTRF/Cavin in HaCat cells.
1544 *PLoS One* **5**, e14157, doi:10.1371/journal.pone.0014157 (2010).

- 1545 94 De Donatis, A. *et al.* Proliferation versus migration in platelet-derived growth
1546 factor signaling: the key role of endocytosis. *J Biol Chem* **283**, 19948-19956,
1547 doi:10.1074/jbc.M709428200 (2008).
- 1548 95 Jastrzebski, K. *et al.* Multiple routes of endocytic internalization of PDGFRbeta
1549 contribute to PDGF-induced STAT3 signaling. *J Cell Sci* **130**, 577-589,
1550 doi:10.1242/jcs.191213 (2017).
- 1551 96 Sadowski, L. *et al.* Dynamin inhibitors impair endocytosis and mitogenic signaling
1552 of PDGF. *Traffic* **14**, 725-736, doi:10.1111/tra.12061 (2013).
- 1553 97 Basagiannis, D. *et al.* VEGF induces signalling and angiogenesis by directing
1554 VEGFR2 internalisation through macropinocytosis. *J Cell Sci* **129**, 4091-4104,
1555 doi:10.1242/jcs.188219 (2016).
- 1556 98 Genet, G. *et al.* Endophilin-A2 dependent VEGFR2 endocytosis promotes
1557 sprouting angiogenesis. *Nat Commun* **10**, 2350, doi:10.1038/s41467-019-10359-x
1558 (2019).
- 1559 99 Nakayama, M. *et al.* Spatial regulation of VEGF receptor endocytosis in
1560 angiogenesis. *Nat Cell Biol* **15**, 249-260, doi:10.1038/ncb2679 (2013).
- 1561 100 Sawamiphak, S. *et al.* Ephrin-B2 regulates VEGFR2 function in developmental and
1562 tumour angiogenesis. *Nature* **465**, 487-491, doi:10.1038/nature08995 (2010).
- 1563 101 Marques, P. E., Grinstein, S. & Freeman, S. A. SnapShot:Macropinocytosis. *Cell*
1564 **169**, 766-766 e761, doi:10.1016/j.cell.2017.04.031 (2017).
- 1565 102 Lin, X. P., Mintern, J. D. & Gleeson, P. A. Macropinocytosis in Different Cell Types:
1566 Similarities and Differences. *Membranes (Basel)* **10**,
1567 doi:10.3390/membranes10080177 (2020).
- 1568 103 Bar-Sagi, D. & Feramisco, J. R. Induction of membrane ruffling and fluid-phase
1569 pinocytosis in quiescent fibroblasts by ras proteins. *Science* **233**, 1061-1068,
1570 doi:10.1126/science.3090687 (1986).
- 1571 104 Porat-Shliom, N., Kloog, Y. & Donaldson, J. G. A unique platform for H-Ras
1572 signaling involving clathrin-independent endocytosis. *Mol Biol Cell* **19**, 765-775,
1573 doi:10.1091/mbc.e07-08-0841 (2008).
- 1574 105 Walsh, A. B. & Bar-Sagi, D. Differential activation of the Rac pathway by Ha-Ras
1575 and K-Ras. *J Biol Chem* **276**, 15609-15615, doi:10.1074/jbc.M010573200 (2001).
- 1576 106 Recouvreux, M. V. & Commisso, C. Macropinocytosis: A Metabolic Adaptation to
1577 Nutrient Stress in Cancer. *Front Endocrinol (Lausanne)* **8**, 261,
1578 doi:10.3389/fendo.2017.00261 (2017).
- 1579 107 Kim, S. M. *et al.* PTEN Deficiency and AMPK Activation Promote Nutrient
1580 Scavenging and Anabolism in Prostate Cancer Cells. *Cancer Discov* **8**, 866-883,
1581 doi:10.1158/2159-8290.CD-17-1215 (2018).
- 1582 108 Jayashankar, V. & Edinger, A. L. Macropinocytosis confers resistance to therapies
1583 targeting cancer anabolism. *Nat Commun* **11**, 1121, doi:10.1038/s41467-020-
1584 14928-3 (2020).
- 1585 **This study establishes necrocytosis as a mechanism of drug resistance,**
1586 **demonstrating its role in supplying amino acids, sugars, fatty acids and**
1587 **nucleotides for biosynthesis, and evidencing that it confers resistance to**
1588 **therapies targeting anabolic pathways in a cell context-dependent manner.**
- 1589 109 Jayashankar, V., Finicle, B. T. & Edinger, A. L. Starving PTEN-deficient prostate
1590 cancer cells thrive under nutrient stress by scavenging corpses for their supper.
1591 *Mol Cell Oncol* **5**, e1472060, doi:10.1080/23723556.2018.1472060 (2018).

- 110 Norris, A. & Grant, B. D. Endosomal microdomains: Formation and function. *Curr Opin Cell Biol* **65**, 86-95, doi:10.1016/j.ceb.2020.02.018 (2020).
- 111 Eichel, K. & von Zastrow, M. Subcellular Organization of GPCR Signaling. *Trends Pharmacol Sci* **39**, 200-208, doi:10.1016/j.tips.2017.11.009 (2018).
- 112 Irannejad, R. & von Zastrow, M. GPCR signaling along the endocytic pathway. *Curr Opin Cell Biol* **27**, 109-116, doi:10.1016/j.ceb.2013.10.003 (2014).
- 113 Jha, A., van Zanten, T. S., Philippe, J. M., Mayor, S. & Lecuit, T. Quantitative Control of GPCR Organization and Signaling by Endocytosis in Epithelial Morphogenesis. *Curr Biol* **28**, 1570-1584 e1576, doi:10.1016/j.cub.2018.03.068 (2018).
Shows that in the *D. melanogaster* embryo, the dynamic partitioning of active GPCRs at the plasma membrane or in plasma membrane invaginations by endocytosis creates platforms for RHO1 signaling and MyoII activation, which regulate epithelial morphogenesis.
- 114 Kerridge, S. *et al.* Modular activation of Rho1 by GPCR signalling imparts polarized myosin II activation during morphogenesis. *Nat Cell Biol* **18**, 261-270, doi:10.1038/ncb3302 (2016).
- 115 Irannejad, R. *et al.* Functional selectivity of GPCR-directed drug action through location bias. *Nat Chem Biol* **13**, 799-806, doi:10.1038/nchembio.2389 (2017).
In this study, the human β 1-adrenergic receptor is shown to induce cAMP signalling from the Golgi apparatus, leading authors to propose 'location bias' as a new principle for achieving functional selectivity of GPCR-directed drug action.
- 116 Godbole, A., Lyga, S., Lohse, M. J. & Calebiro, D. Internalized TSH receptors en route to the TGN induce local Gs-protein signaling and gene transcription. *Nat Commun* **8**, 443, doi:10.1038/s41467-017-00357-2 (2017).
- 117 Boivin, B., Vaniotis, G., Allen, B. G. & Hebert, T. E. G protein-coupled receptors in and on the cell nucleus: a new signaling paradigm? *J Recept Signal Transduct Res* **28**, 15-28, doi:10.1080/10799890801941889 (2008).
- 118 Calebiro, D. *et al.* Persistent cAMP-signals triggered by internalized G-protein-coupled receptors. *PLoS Biol* **7**, e1000172, doi:10.1371/journal.pbio.1000172 (2009).
- 119 Inda, C. *et al.* Different cAMP sources are critically involved in G protein-coupled receptor CRHR1 signaling. *J Cell Biol* **214**, 181-195, doi:10.1083/jcb.201512075 (2016).
- 120 Lazar, A. M. *et al.* G protein-regulated endocytic trafficking of adenylyl cyclase type 9. *Elife* **9**, doi:10.7554/eLife.58039 (2020).
- 121 Tsvetanova, N. G. & von Zastrow, M. Spatial encoding of cyclic AMP signaling specificity by GPCR endocytosis. *Nat Chem Biol* **10**, 1061-1065, doi:10.1038/nchembio.1665 (2014).
- 122 Ritter, S. L. & Hall, R. A. Fine-tuning of GPCR activity by receptor-interacting proteins. *Nat Rev Mol Cell Biol* **10**, 819-830, doi:10.1038/nrm2803 (2009).
- 123 Weinberg, Z. Y. & Puthenveedu, M. A. Regulation of G protein-coupled receptor signaling by plasma membrane organization and endocytosis. *Traffic* **20**, 121-129, doi:10.1111/tra.12628 (2019).

1637 124 Heuninck, J. *et al.* Context-Dependent Signaling of CXC Chemokine Receptor 4
1638 and Atypical Chemokine Receptor 3. *Mol Pharmacol* **96**, 778-793,
1639 doi:10.1124/mol.118.115477 (2019).

1640 125 Hauser, A. S. *et al.* Pharmacogenomics of GPCR Drug Targets. *Cell* **172**, 41-54 e19,
1641 doi:10.1016/j.cell.2017.11.033 (2018).

1642 126 Retamal, J. S., Ramirez-Garcia, P. D., Shenoy, P. A., Poole, D. P. & Veldhuis, N. A.
1643 Internalized GPCRs as Potential Therapeutic Targets for the Management of Pain.
1644 *Front Mol Neurosci* **12**, 273, doi:10.3389/fnmol.2019.00273 (2019).

1645 127 Jimenez-Vargas, N. N. *et al.* Protease-activated receptor-2 in endosomes signals
1646 persistent pain of irritable bowel syndrome. *Proc Natl Acad Sci U S A* **115**, E7438-
1647 E7447, doi:10.1073/pnas.1721891115 (2018).

1648 128 Jimenez-Vargas, N. N. *et al.* Endosomal signaling of delta opioid receptors is an
1649 endogenous mechanism and therapeutic target for relief from inflammatory
1650 pain. *Proc Natl Acad Sci U S A* **117**, 15281-15292, doi:10.1073/pnas.2000500117
1651 (2020).

1652 129 Ramirez-Garcia, P. D. *et al.* A pH-responsive nanoparticle targets the neurokinin 1
1653 receptor in endosomes to prevent chronic pain. *Nat Nanotechnol* **14**, 1150-1159,
1654 doi:10.1038/s41565-019-0568-x (2019).

1655 130 Arakaki, A. K. S., Pan, W. A. & Trejo, J. GPCRs in Cancer: Protease-Activated
1656 Receptors, Endocytic Adaptors and Signaling. *Int J Mol Sci* **19**,
1657 doi:10.3390/ijms19071886 (2018).

1658 131 Dorsam, R. T. & Gutkind, J. S. G-protein-coupled receptors and cancer. *Nat Rev*
1659 *Cancer* **7**, 79-94, doi:10.1038/nrc2069 (2007).

1660 132 Gad, A. A. & Balenga, N. The Emerging Role of Adhesion GPCRs in Cancer. *ACS*
1661 *Pharmacol Transl Sci* **3**, 29-42, doi:10.1021/acsptsci.9b00093 (2020).

1662 133 Usman, S., Khawer, M., Rafique, S., Naz, Z. & Saleem, K. The current status of
1663 anti-GPCR drugs against different cancers. *Journal of Pharmaceutical Analysis* **10**,
1664 517-521, doi:https://doi.org/10.1016/j.jpha.2020.01.001 (2020).

1665 134 Lavoie, H., Gagnon, J. & Therrien, M. ERK signalling: a master regulator of cell
1666 behaviour, life and fate. *Nat Rev Mol Cell Biol* **21**, 607-632, doi:10.1038/s41580-
1667 020-0255-7 (2020).

1668 135 Schiefermeier, N., Teis, D. & Huber, L. A. Endosomal signaling and cell migration.
1669 *Curr Opin Cell Biol* **23**, 615-620, doi:10.1016/j.ceb.2011.04.001 (2011).

1670 136 Matsubayashi, Y., Ebisuya, M., Honjoh, S. & Nishida, E. ERK activation propagates
1671 in epithelial cell sheets and regulates their migration during wound healing. *Curr*
1672 *Biol* **14**, 731-735, doi:10.1016/j.cub.2004.03.060 (2004).

1673 137 Aoki, K. *et al.* Propagating Wave of ERK Activation Orients Collective Cell
1674 Migration. *Dev Cell* **43**, 305-317 e305, doi:10.1016/j.devcel.2017.10.016 (2017).

1675 138 Malinverno, C. *et al.* Endocytic reawakening of motility in jammed epithelia. *Nat*
1676 *Mater* **16**, 587-596, doi:10.1038/nmat4848 (2017).

1677 139 Villasenor, R., Nonaka, H., Del Conte-Zerial, P., Kalaidzidis, Y. & Zerial, M.
1678 Regulation of EGFR signal transduction by analogue-to-digital conversion in
1679 endosomes. *Elife* **4**, doi:10.7554/eLife.06156 (2015).

1680 140 Cullen, P. J. & Steinberg, F. To degrade or not to degrade: mechanisms and
1681 significance of endocytic recycling. *Nat Rev Mol Cell Biol* **19**, 679-696,
1682 doi:10.1038/s41580-018-0053-7 (2018).

- 141 Zerial, M. & McBride, H. Rab proteins as membrane organizers. *Nat Rev Mol Cell Biol* **2**, 107-117, doi:10.1038/35052055 (2001).
- 142 Tebar, F., Enrich, C., Rentero, C. & Grewal, T. GTPases Rac1 and Ras Signaling from Endosomes. *Prog Mol Subcell Biol* **57**, 65-105, doi:10.1007/978-3-319-96704-2_3 (2018).
- 143 Horiuchi, H. *et al.* A novel Rab5 GDP/GTP exchange factor complexed to Rabaptin-5 links nucleotide exchange to effector recruitment and function. *Cell* **90**, 1149-1159, doi:10.1016/s0092-8674(00)80380-3 (1997).
- 144 Christoforidis, S. *et al.* Phosphatidylinositol-3-OH kinases are Rab5 effectors. *Nat Cell Biol* **1**, 249-252, doi:10.1038/12075 (1999).
- 145 Christoforidis, S., McBride, H. M., Burgoyne, R. D. & Zerial, M. The Rab5 effector EEA1 is a core component of endosome docking. *Nature* **397**, 621-625, doi:10.1038/17618 (1999).
- 146 Cezanne, A., Lauer, J., Solomatina, A., Sbalzarini, I. F. & Zerial, M. A non-linear system patterns Rab5 GTPase on the membrane. *Elife* **9**, doi:10.7554/eLife.54434 (2020).
- Using an in vitro reconstituted system with lipid bilayers, this study shows that positive feedback regulatory loops control RAB5 recruitment and activation on early endosomes, determining its patterning on endosomal membranes.**
- 147 Murray, J. T., Panaretou, C., Stenmark, H., Miaczynska, M. & Backer, J. M. Role of Rab5 in the recruitment of hVps34/p150 to the early endosome. *Traffic* **3**, 416-427, doi:10.1034/j.1600-0854.2002.30605.x (2002).
- 148 Edler, E. & Stein, M. Probing the druggability of membrane-bound Rab5 by molecular dynamics simulations. *J Enzyme Inhib Med Chem* **32**, 434-443, doi:10.1080/14756366.2016.1260564 (2017).
- 149 Munzberg, E. & Stein, M. Structure and Dynamics of Mono- vs. Doubly Lipidated Rab5 in Membranes. *Int J Mol Sci* **20**, doi:10.3390/ijms20194773 (2019).
- 150 Bucci, C. *et al.* Co-operative regulation of endocytosis by three Rab5 isoforms. *FEBS Lett* **366**, 65-71, doi:10.1016/0014-5793(95)00477-q (1995).
- 151 Wainszelbaum, M. J., Proctor, B. M., Pontow, S. E., Stahl, P. D. & Barbieri, M. A. IL4/PGE2 induction of an enlarged early endosomal compartment in mouse macrophages is Rab5-dependent. *Exp Cell Res* **312**, 2238-2251, doi:10.1016/j.yexcr.2006.03.025 (2006).
- 152 Chen, P. I., Kong, C., Su, X. & Stahl, P. D. Rab5 isoforms differentially regulate the trafficking and degradation of epidermal growth factor receptors. *J Biol Chem* **284**, 30328-30338, doi:10.1074/jbc.M109.034546 (2009).
- 153 Bhattacharya, M. *et al.* IL-6 and IL-12 specifically regulate the expression of Rab5 and Rab7 via distinct signaling pathways. *EMBO J* **25**, 2878-2888, doi:10.1038/sj.emboj.7601170 (2006).
- 154 Goryachev, A. B. & Pokhilko, A. V. Dynamics of Cdc42 network embodies a Turing-type mechanism of yeast cell polarity. *FEBS Lett* **582**, 1437-1443, doi:10.1016/j.febslet.2008.03.029 (2008).
- 155 Witte, K., Strickland, D. & Glotzer, M. Cell cycle entry triggers a switch between two modes of Cdc42 activation during yeast polarization. *Elife* **6**, doi:10.7554/eLife.26722 (2017).

1728 156 Zhou, Y. *et al.* Lipid-Sorting Specificity Encoded in K-Ras Membrane Anchor
1729 Regulates Signal Output. *Cell* **168**, 239-251 e216, doi:10.1016/j.cell.2016.11.059
1730 (2017).

1731 **Demonstration that clustering of K-RAS at the plasma membrane leads to the**
1732 **assembly of specific phospholipids into nanoclusters determining K-RAS**
1733 **signalling output.**

1734 157 Halatek, J., Brauns, F. & Frey, E. Self-organization principles of intracellular
1735 pattern formation. *Philos Trans R Soc Lond B Biol Sci* **373**,
1736 doi:10.1098/rstb.2017.0107 (2018).

1737 158 Goryachev, A. B. & Leda, M. Autoactivation of small GTPases by the GEF-effector
1738 positive feedback modules. *F1000Res* **8**, doi:10.12688/f1000research.20003.1
1739 (2019).

1740 159 Mellman, I. & Nelson, W. J. Coordinated protein sorting, targeting and
1741 distribution in polarized cells. *Nat Rev Mol Cell Biol* **9**, 833-845, doi:nrm2525
1742 [pii]10.1038/nrm2525 (2008).

1743 160 Datta, A., Bryant, D. M. & Mostov, K. E. Molecular regulation of lumen
1744 morphogenesis. *Curr Biol* **21**, R126-136, doi:10.1016/j.cub.2010.12.003 (2011).

1745 161 Jewett, C. E. & Prekeris, R. Insane in the apical membrane: Trafficking events
1746 mediating apicobasal epithelial polarity during tube morphogenesis. *Traffic*,
1747 doi:10.1111/tra.12579 (2018).

1748 162 Overeem, A. W., Bryant, D. M. & van, I. S. C. Mechanisms of apical-basal axis
1749 orientation and epithelial lumen positioning. *Trends Cell Biol* **25**, 476-485,
1750 doi:10.1016/j.tcb.2015.04.002 (2015).

1751 163 Gandalovicova, A., Vomastek, T., Rosel, D. & Brabek, J. Cell polarity signaling in
1752 the plasticity of cancer cell invasiveness. *Oncotarget* **7**, 25022-25049,
1753 doi:10.18632/oncotarget.7214 (2016).

1754 164 Roman-Fernandez, A. & Bryant, D. M. Complex Polarity: Building Multicellular
1755 Tissues Through Apical Membrane Traffic. *Traffic* **17**, 1244-1261,
1756 doi:10.1111/tra.12417 (2016).

1757 **This study shows that the unusual phospholipid PI(3,4)P₂, together with**
1758 **PI(4,5)P₂, is found apically enriched during the early phases of lumen**
1759 **formation and controls polarity establishment.**

1760 165 West, J. J. & Harris, T. J. Cadherin Trafficking for Tissue Morphogenesis: Control
1761 and Consequences. *Traffic* **17**, 1233-1243, doi:10.1111/tra.12407 (2016).

1762 166 Mrozowska, P. S. & Fukuda, M. Regulation of podocalyxin trafficking by Rab small
1763 GTPases in 2D and 3D epithelial cell cultures. *J Cell Biol* **213**, 355-369,
1764 doi:10.1083/jcb.201512024 (2016).

1765 167 Diaz-Diaz, C., Baonza, G. & Martin-Belmonte, F. The vertebrate epithelial apical
1766 junctional complex: Dynamic interplay between Rho GTPase activity and cell
1767 polarization processes. *Biochim Biophys Acta Biomembr* **1862**, 183398,
1768 doi:10.1016/j.bbamem.2020.183398 (2020).

1769 168 Bryant, D. M. & Mostov, K. E. From cells to organs: building polarized tissue. *Nat*
1770 *Rev Mol Cell Biol* **9**, 887-901, doi:10.1038/nrm2523 (2008).

1771 169 Nelson, W. J. Adaptation of core mechanisms to generate cell polarity. *Nature*
1772 **422**, 766-774, doi:10.1038/nature01602 (2003).

1773 170 Schluter, M. A. & Margolis, B. Apicobasal polarity in the kidney. *Exp Cell Res* **318**,
1774 1033-1039, doi:10.1016/j.yexcr.2012.02.028 (2012).

1775 171 Bryant, D. M. *et al.* A molecular network for de novo generation of the apical
1776 surface and lumen. *Nat Cell Biol* **12**, 1035-1045, doi:10.1038/ncb2106 (2010).
1777 172 Mangan, A. J. *et al.* Cingulin and actin mediate midbody-dependent apical lumen
1778 formation during polarization of epithelial cells. *Nat Commun* **7**, 12426,
1779 doi:10.1038/ncomms12426 (2016).
1780 173 Su, T. *et al.* A kinase cascade leading to Rab11-FIP5 controls transcytosis of the
1781 polymeric immunoglobulin receptor. *Nat Cell Biol* **12**, 1143-1153,
1782 doi:10.1038/ncb2118 (2010).
1783 174 Klinkert, K., Rocancourt, M., Houdusse, A. & Echard, A. Rab35 GTPase couples
1784 cell division with initiation of epithelial apico-basal polarity and lumen opening.
1785 *Nat Commun* **7**, 11166, doi:10.1038/ncomms11166 (2016).
1786 175 Kinoshita, R., Homma, Y. & Fukuda, M. Rab35-GEFs, DENND1A and folliculin
1787 differentially regulate podocalyxin trafficking in two- and three-dimensional
1788 epithelial cell cultures. *J Biol Chem*, doi:10.1074/jbc.RA119.011646 (2020).
1789 176 Willenborg, C. *et al.* Interaction between FIP5 and SNX18 regulates epithelial
1790 lumen formation. *J Cell Biol* **195**, 71-86, doi:10.1083/jcb.201011112 (2011).
1791 177 Roman-Fernandez, A. *et al.* The phospholipid PI(3,4)P2 is an apical identity
1792 determinant. *Nat Commun* **9**, 5041, doi:10.1038/s41467-018-07464-8 (2018).
1793 178 Martin-Belmonte, F. *et al.* PTEN-mediated apical segregation of
1794 phosphoinositides controls epithelial morphogenesis through Cdc42. *Cell* **128**,
1795 383-397, doi:10.1016/j.cell.2006.11.051 (2007).
1796 179 Bisi, S. *et al.* IRSp53 controls plasma membrane shape and polarized transport at
1797 the nascent lumen in epithelial tubules. *Nat Commun* **11**, 3516,
1798 doi:10.1038/s41467-020-17091-x (2020).
1799 **The I-BAR containing protein, IRSP53, is shown to be an early apical**
1800 **determinant that binds to RAB35 and facilitates the transport and the**
1801 **anchoring of podocalyxin to the apical membrane initiation site, where it also**
1802 **controls the integrity and the shape of the plasma membrane.**
1803 180 Zhang, Y. *et al.* Biomimetic niches reveal the minimal cues to trigger apical lumen
1804 formation in single hepatocytes. *Nat Mater*, doi:10.1038/s41563-020-0662-3
1805 (2020).
1806 **An elegant study that shows how the density and localization of cadherins,**
1807 **along the initial cell-to-cell contact, represent the minimal molecular and**
1808 **physical cues to trigger the development of asymmetric lateral hemilumen in**
1809 **hepatocytes.**
1810 181 Li, Q. *et al.* Extracellular matrix scaffolding guides lumen elongation by inducing
1811 anisotropic intercellular mechanical tension. *Nat Cell Biol* **18**, 311-318,
1812 doi:10.1038/ncb3310 (2016).
1813 182 Ferrari, A., Veligodskiy, A., Berge, U., Lucas, M. S. & Kroschewski, R. ROCK-
1814 mediated contractility, tight junctions and channels contribute to the conversion
1815 of a preapical patch into apical surface during isochoric lumen initiation. *J Cell Sci*
1816 **121**, 3649-3663, doi:10.1242/jcs.018648 (2008).
1817 183 Saito, Y., Desai, R. R. & Muthuswamy, S. K. Reinterpreting polarity and cancer:
1818 The changing landscape from tumor suppression to tumor promotion. *Biochim*
1819 *Biophys Acta Rev Cancer* **1869**, 103-116, doi:10.1016/j.bbcan.2017.12.001
1820 (2018).

- 1821 184 Vladar, E. K., Antic, D. & Axelrod, J. D. Planar cell polarity signaling: the
1822 developing cell's compass. *Cold Spring Harb Perspect Biol* **1**, a002964,
1823 doi:10.1101/cshperspect.a002964 (2009).
- 1824 185 Simons, M. & Mlodzik, M. Planar cell polarity signaling: from fly development to
1825 human disease. *Annu Rev Genet* **42**, 517-540,
1826 doi:10.1146/annurev.genet.42.110807.091432 (2008).
- 1827 186 Strutt, H. & Strutt, D. Asymmetric localisation of planar polarity proteins:
1828 Mechanisms and consequences. *Semin Cell Dev Biol* **20**, 957-963,
1829 doi:10.1016/j.semcdb.2009.03.006 (2009).
- 1830 187 Maung, S. M. & Jenny, A. Planar cell polarity in Drosophila. *Organogenesis* **7**,
1831 165-179, doi:10.4161/org.7.3.18143 (2011).
- 1832 188 Xie, Y., Miao, H. & Blankenship, J. T. Membrane trafficking in morphogenesis and
1833 planar polarity. *Traffic*, doi:10.1111/tra.12580 (2018).
- 1834 189 Butler, M. T. & Wallingford, J. B. Planar cell polarity in development and disease.
1835 *Nat Rev Mol Cell Biol* **18**, 375-388, doi:10.1038/nrm.2017.11 (2017).
- 1836 190 Voiculescu, O., Bertocchini, F., Wolpert, L., Keller, R. E. & Stern, C. D. The amniote
1837 primitive streak is defined by epithelial cell intercalation before gastrulation.
1838 *Nature* **449**, 1049-1052, doi:10.1038/nature06211 (2007).
- 1839 191 Takeichi, M. Dynamic contacts: rearranging adherens junctions to drive epithelial
1840 remodelling. *Nat Rev Mol Cell Biol* **15**, 397-410, doi:10.1038/nrm3802 (2014).
- 1841 192 Harris, T. J. C. Sculpting epithelia with planar polarized actomyosin networks:
1842 Principles from Drosophila. *Semin Cell Dev Biol* **81**, 54-61,
1843 doi:10.1016/j.semcdb.2017.07.042 (2018).
- 1844 193 Pare, A. C. & Zallen, J. A. Cellular, molecular, and biophysical control of epithelial
1845 cell intercalation. *Curr Top Dev Biol* **136**, 167-193,
1846 doi:10.1016/bs.ctdb.2019.11.014 (2020).
- 1847 194 Truong Quang, B. A., Mani, M., Markova, O., Lecuit, T. & Lenne, P. F. Principles of
1848 E-cadherin supramolecular organization in vivo. *Curr Biol* **23**, 2197-2207,
1849 doi:10.1016/j.cub.2013.09.015 (2013).
- 1850 195 Levayer, R., Pelissier-Monier, A. & Lecuit, T. Spatial regulation of Dia and Myosin-
1851 II by RhoGEF2 controls initiation of E-cadherin endocytosis during epithelial
1852 morphogenesis. *Nat Cell Biol* **13**, 529-540, doi:10.1038/ncb2224 (2011).
- 1853 196 Pope, K. L. & Harris, T. J. Control of cell flattening and junctional remodeling
1854 during squamous epithelial morphogenesis in Drosophila. *Development* **135**,
1855 2227-2238, doi:10.1242/dev.019802 (2008).
- 1856 197 Cavanaugh, K. E., Staddon, M. F., Munro, E., Banerjee, S. & Gardel, M. L. RhoA
1857 Mediates Epithelial Cell Shape Changes via Mechanosensitive Endocytosis. *Dev*
1858 *Cell*, doi:10.1016/j.devcel.2019.12.002 (2019).
- 1859 **This study (together with work by Sumi et al. (2018)) illustrates how the**
1860 **pulsating activity of RHOA-mediated contractility, in model epithelial tissues,**
1861 **leads to fluctuation in junctional length, and that the shortening of the junction**
1862 **requires formin-mediated E-cadherin clustering and dynamin-dependent**
1863 **endocytosis.**
- 1864 198 Sumi, A. et al. Adherens Junction Length during Tissue Contraction Is Controlled
1865 by the Mechanosensitive Activity of Actomyosin and Junctional Recycling. *Dev*
1866 *Cell* **47**, 453-463 e453, doi:10.1016/j.devcel.2018.10.025 (2018).

Shows that during amnioserosa contraction in *D. melanogaster*, adherens junctions reduce their length in coordination with the shrinkage of apical cell area, maintaining a nearly constant junctional straightness, which is ensured by the endocytic machinery that removes excess plasma membrane.

- 199 Miao, H., Vanderleest, T. E., Jewett, C. E., Loerke, D. & Blankenship, J. T. Cell ratcheting through the Sbf RabGEF directs force balancing and stepped apical constriction. *J Cell Biol* **218**, 3845-3860, doi:10.1083/jcb.201905082 (2019).

- 200 Jewett, C. E. *et al.* Planar polarized Rab35 functions as an oscillatory ratchet during cell intercalation in the Drosophila epithelium. *Nat Commun* **8**, 476, doi:10.1038/s41467-017-00553-0 (2017).

By examining RAB protein distributions during cell intercalation in *D. melanogaster* epithelial tissue remodelling, RAB35-mediated endocytosis of plasma membrane at junctions is found to serve as a unique ratcheting device that directs progressive interface contraction.

- 201 Kouranti, I., Sachse, M., Arouche, N., Goud, B. & Echard, A. Rab35 regulates an endocytic recycling pathway essential for the terminal steps of cytokinesis. *Curr Biol* **16**, 1719-1725, doi:10.1016/j.cub.2006.07.020 (2006).

- 202 Corallino, S. *et al.* A RAB35-p85/PI3K axis controls oscillatory apical protrusions required for efficient chemotactic migration. *Nat Commun* **9**, 1475, doi:10.1038/s41467-018-03571-8 (2018).

Demonstration that RAB35 plays a critical role in regulating the formation of oscillatory, apical circular ruffles that act as steering devices during chemotaxis and promote efficient migration and invasion in breast cancer.

- 203 Wheeler, D. B., Zoncu, R., Root, D. E., Sabatini, D. M. & Sawyers, C. L. Identification of an oncogenic RAB protein. *Science* **350**, 211-217, doi:10.1126/science.aaa4903 (2015).

- 204 Shaughnessy, R. & Echard, A. Rab35 GTPase and cancer: Linking membrane trafficking to tumorigenesis. *Traffic* **19**, 247-252, doi:10.1111/tra.12546 (2018).

- 205 Rainero, E. Extracellular matrix internalization links nutrient signalling to invasive migration. *Int J Exp Pathol* **99**, 4-9, doi:10.1111/iep.12265 (2018).

- 206 Yong, C. Q. Y. & Tang, B. L. Cancer-driving mutations and variants of components of the membrane trafficking core machinery. *Life Sci* **264**, 118662, doi:10.1016/j.lfs.2020.118662 (2021).

- 207 Bendris, N. & Schmid, S. L. Endocytosis, Metastasis and Beyond: Multiple Facets of SNX9. *Trends Cell Biol* **27**, 189-200, doi:10.1016/j.tcb.2016.11.001 (2017).

- 208 Bisi, S. *et al.* Membrane and actin dynamics interplay at lamellipodia leading edge. *Curr Opin Cell Biol* **25**, 565-573, doi:10.1016/j.ceb.2013.04.001 (2013).

- 209 Caswell, P. T., Vadrevu, S. & Norman, J. C. Integrins: masters and slaves of endocytic transport. *Nat Rev Mol Cell Biol* **10**, 843-853, doi:10.1038/nrm2799 (2009).

- 210 De Franceschi, N., Hamidi, H., Alanko, J., Sahgal, P. & Ivaska, J. Integrin traffic - the update. *J Cell Sci* **128**, 839-852, doi:10.1242/jcs.161653 (2015).

- 211 Montell, D. J., Yoon, W. H. & Starz-Gaiano, M. Group choreography: mechanisms orchestrating the collective movement of border cells. *Nat Rev Mol Cell Biol* **13**, 631-645, doi:10.1038/nrm3433 (2012).

- 1912 212 Cheung, K. J. *et al.* Polyclonal breast cancer metastases arise from collective
1913 dissemination of keratin 14-expressing tumor cell clusters. *Proc Natl Acad Sci U S*
1914 *A* **113**, E854-863, doi:10.1073/pnas.1508541113 (2016).
- 1915 213 Cheung, K. J., Gabrielson, E., Werb, Z. & Ewald, A. J. Collective invasion in breast
1916 cancer requires a conserved basal epithelial program. *Cell* **155**, 1639-1651,
1917 doi:10.1016/j.cell.2013.11.029 (2013).
- 1918 214 Hakim, V. & Silberzan, P. Collective cell migration: a physics perspective. *Rep*
1919 *Prog Phys* **80**, 076601, doi:10.1088/1361-6633/aa65ef (2017).
- 1920 215 Tambe, D. T. *et al.* Collective cell guidance by cooperative intercellular forces.
1921 *Nat Mater* **10**, 469-475, doi:10.1038/nmat3025 (2011).
- 1922 216 Peglion, F., Lense, F. & Etienne-Manneville, S. Adherens junction treadmilling
1923 during collective migration. *Nat Cell Biol* **16**, 639-651, doi:10.1038/ncb2985
1924 (2014).
- 1925 217 Theveneau, E. & Mayor, R. Neural crest delamination and migration: from
1926 epithelium-to-mesenchyme transition to collective cell migration. *Dev Biol* **366**,
1927 34-54, doi:10.1016/j.ydbio.2011.12.041 (2012).
- 1928 218 Lin, M. E., Herr, D. R. & Chun, J. Lysophosphatidic acid (LPA) receptors: signaling
1929 properties and disease relevance. *Prostaglandins Other Lipid Mediat* **91**, 130-
1930 138, doi:10.1016/j.prostaglandins.2009.02.002 (2010).
- 1931 219 Kuriyama, S. *et al.* In vivo collective cell migration requires an LPAR2-dependent
1932 increase in tissue fluidity. *J Cell Biol* **206**, 113-127, doi:10.1083/jcb.201402093
1933 (2014).
- 1934 220 Muinonen-Martin, A. J. *et al.* Melanoma cells break down LPA to establish local
1935 gradients that drive chemotactic dispersal. *PLoS Biol* **12**, e1001966,
1936 doi:10.1371/journal.pbio.1001966 (2014).
- 1937 221 Juin, A. *et al.* N-WASP Control of LPAR1 Trafficking Establishes Response to Self-
1938 Generated LPA Gradients to Promote Pancreatic Cancer Cell Metastasis. *Dev Cell*
1939 **51**, 431-445 e437, doi:10.1016/j.devcel.2019.09.018 (2019).
- 1940 **Demonstration that the promoter of actin nucleation, N-WASP (WASL), drives**
1941 **the trafficking of LPA receptors to control cellular responses to self-generated**
1942 **gradients and to enhance metastatic spreading of pancreatic ductal**
1943 **carcinomas.**
- 1944 222 Leyton-Puig, D. *et al.* Flat clathrin lattices are dynamic actin-controlled hubs for
1945 clathrin-mediated endocytosis and signalling of specific receptors. *Nat Commun*
1946 **8**, 16068, doi:10.1038/ncomms16068 (2017).
- 1947 223 Park, J. A. *et al.* Unjamming and cell shape in the asthmatic airway epithelium.
1948 *Nat Mater* **14**, 1040-1048, doi:10.1038/nmat4357 (2015).
- 1949 **A seminal paper showing the physical principles governing the transition from**
1950 **a solid, jammed to a collectively-moving and unjammed pseudostratified**
1951 **human bronchial epithelial system from asthmatic patients.**
- 1952 224 Sadati, M., Nourhani, A., Fredberg, J. J. & Taheri Qazvini, N. Glass-like dynamics
1953 in the cell and in cellular collectives. *Wiley Interdiscip Rev Syst Biol Med* **6**, 137-
1954 149, doi:10.1002/wsbm.1258 (2014).
- 1955 225 Sadati, M., Taheri Qazvini, N., Krishnan, R., Park, C. Y. & Fredberg, J. J. Collective
1956 migration and cell jamming. *Differentiation* **86**, 121-125,
1957 doi:10.1016/j.diff.2013.02.005 (2013).

- 1958 226 Park, J. A., Atia, L., Mitchel, J. A., Fredberg, J. J. & Butler, J. P. Collective migration
1959 and cell jamming in asthma, cancer and development. *J Cell Sci* **129**, 3375-3383,
1960 doi:10.1242/jcs.187922 (2016).
- 1961 227 Atia, L. *et al.* Geometric constraints during epithelial jamming. *Nat Phys* **14**, 613-
1962 620, doi:10.1038/s41567-018-0089-9 (2018).
- 1963 228 Bi, D., Yang, X., Marchetti, M. C. & Manning, M. L. Motility-Driven Glass and
1964 Jamming Transitions in Biological Tissues. *Physical Review X* **6**, 021011 (2016).
- 1965 229 Garcia, S. *et al.* Physics of active jamming during collective cellular motion in a
1966 monolayer. *Proc Natl Acad Sci U S A* **112**, 15314-15319,
1967 doi:10.1073/pnas.1510973112 (2015).
- 1968 230 de Beco, S., Amblard, F. & Coscoy, S. New insights into the regulation of E-
1969 cadherin distribution by endocytosis. *International review of cell and molecular*
1970 *biology* **295**, 63-108, doi:10.1016/B978-0-12-394306-4.00008-3 (2012).
- 1971 231 Palamidessi, A. *et al.* Unjamming overcomes kinetic and proliferation arrest in
1972 terminally differentiated cells and promotes collective motility of carcinoma. *Nat*
1973 *Mater* **18**, 1252-1263, doi:10.1038/s41563-019-0425-1 (2019).
- 1974 232 Ilin, O. *et al.* Cell-cell adhesion and 3D matrix confinement determine jamming
1975 transitions in breast cancer invasion. *Nat Cell Biol* **22**, 1103-1115,
1976 doi:10.1038/s41556-020-0552-6 (2020).
- 1977 **In this work the authors show that cell crowding induced by matrix**
1978 **confinement and cell-cell adhesion modulate the jamming transition during**
1979 **breast cancer invasion.**
- 1980 233 Mongera, A. *et al.* A fluid-to-solid jamming transition underlies vertebrate body
1981 axis elongation. *Nature* **561**, 401-405, doi:10.1038/s41586-018-0479-2 (2018).
- 1982 234 Mitchel, J. A. *et al.* In primary airway epithelial cells, the unjamming transition is
1983 distinct from the epithelial-to-mesenchymal transition. *Nat Commun* **11**, 5053,
1984 doi:10.1038/s41467-020-18841-7 (2020).
- 1985 235 Greenburg, G. & Hay, E. D. Epithelia suspended in collagen gels can lose polarity
1986 and express characteristics of migrating mesenchymal cells. *J Cell Biol* **95**, 333-
1987 339, doi:10.1083/jcb.95.1.333 (1982).
- 1988 236 Nieto, M. A., Huang, R. Y., Jackson, R. A. & Thiery, J. P. EMT: 2016. *Cell* **166**, 21-45,
1989 doi:10.1016/j.cell.2016.06.028 (2016).
- 1990 237 Aiello, N. M. & Kang, Y. Context-dependent EMT programs in cancer metastasis. *J*
1991 *Exp Med* **216**, 1016-1026, doi:10.1084/jem.20181827 (2019).
- 1992 238 Bakir, B., Chiarella, A. M., Pitarresi, J. R. & Rustgi, A. K. EMT, MET, Plasticity, and
1993 Tumor Metastasis. *Trends Cell Biol* **30**, 764-776, doi:10.1016/j.tcb.2020.07.003
1994 (2020).
- 1995 239 Pastushenko, I. & Blanpain, C. EMT Transition States during Tumor Progression
1996 and Metastasis. *Trends Cell Biol* **29**, 212-226, doi:10.1016/j.tcb.2018.12.001
1997 (2019).
- 1998 240 Dongre, A. & Weinberg, R. A. New insights into the mechanisms of epithelial-
1999 mesenchymal transition and implications for cancer. *Nat Rev Mol Cell Biol* **20**, 69-
2000 84, doi:10.1038/s41580-018-0080-4 (2019).
- 2001 241 Mani, S. A. *et al.* The epithelial-mesenchymal transition generates cells with
2002 properties of stem cells. *Cell* **133**, 704-715, doi:10.1016/j.cell.2008.03.027 (2008).
- 2003 242 van Staalduinen, J., Baker, D., Ten Dijke, P. & van Dam, H. Epithelial-
2004 mesenchymal-transition-inducing transcription factors: new targets for tackling

2005 chemoresistance in cancer? *Oncogene* **37**, 6195-6211, doi:10.1038/s41388-018-
2006 0378-x (2018).

2007 243 Shibue, T. & Weinberg, R. A. EMT, CSCs, and drug resistance: the mechanistic link
2008 and clinical implications. *Nat Rev Clin Oncol* **14**, 611-629,
2009 doi:10.1038/nrclinonc.2017.44 (2017).

2010 244 Corallino, S., Malabarba, M. G., Zobel, M., Di Fiore, P. P. & Scita, G. Epithelial-to-
2011 Mesenchymal Plasticity Harnesses Endocytic Circuitries. *Front Oncol* **5**, 45,
2012 doi:10.3389/fonc.2015.00045 (2015).

2013 245 Yang, J. *et al.* Guidelines and definitions for research on epithelial-mesenchymal
2014 transition. *Nat Rev Mol Cell Biol* **21**, 341-352, doi:10.1038/s41580-020-0237-9
2015 (2020).

2016 246 Stemmler, M. P., Eccles, R. L., Brabletz, S. & Brabletz, T. Non-redundant functions
2017 of EMT transcription factors. *Nat Cell Biol* **21**, 102-112, doi:10.1038/s41556-018-
2018 0196-y (2019).

2019 247 Skrypek, N., Goossens, S., De Smedt, E., Vandamme, N. & Berx, G. Epithelial-to-
2020 Mesenchymal Transition: Epigenetic Reprogramming Driving Cellular Plasticity.
2021 *Trends Genet* **33**, 943-959, doi:10.1016/j.tig.2017.08.004 (2017).

2022 248 Lamouille, S., Xu, J. & Derynck, R. Molecular mechanisms of epithelial-
2023 mesenchymal transition. *Nat Rev Mol Cell Biol* **15**, 178-196,
2024 doi:10.1038/nrm3758 (2014).

2025 249 Pastushenko, I. *et al.* Identification of the tumour transition states occurring
2026 during EMT. *Nature* **556**, 463-468, doi:10.1038/s41586-018-0040-3 (2018).

2027 **The first in vivo demonstration that, in tumours, subpopulations can be**
2028 **identified which exhibit all different EMT stages, from epithelial to**
2029 **mesenchymal, through intermediate different hybrid states.**

2030 250 Aiello, N. M. *et al.* EMT Subtype Influences Epithelial Plasticity and Mode of Cell
2031 Migration. *Dev Cell* **45**, 681-695 e684, doi:10.1016/j.devcel.2018.05.027 (2018).

2032 **Through an in vivo approach, in a mouse model of pancreatic ductal**
2033 **adenocarcinoma, it is shown that EMT can proceed through endocytosis rather**
2034 **than transcriptional reprogramming, leading to a partial EMT phenotype.**

2035 251 Reichert, M. *et al.* Regulation of Epithelial Plasticity Determines Metastatic
2036 Organotropism in Pancreatic Cancer. *Dev Cell* **45**, 696-711 e698,
2037 doi:10.1016/j.devcel.2018.05.025 (2018).

2038 **By using several mouse models of pancreatic ductal adenocarcinoma, the**
2039 **authors show that the organotropic metastatic preference is a function of the**
2040 **degree of EMT (full blown EMT vs. partial EMT).**

2041 252 Vieira, A. V., Lamaze, C. & Schmid, S. L. Control of EGF receptor signaling by
2042 clathrin-mediated endocytosis. *Science* **274**, 2086-2089,
2043 doi:10.1126/science.274.5295.2086 (1996).

2044 253 Khan, M. N., Savoie, S., Bergeron, J. J. & Posner, B. I. Characterization of rat liver
2045 endosomal fractions. In vivo activation of insulin-stimulable receptor kinase in
2046 these structures. *J Biol Chem* **261**, 8462-8472 (1986).

2047 254 Lai, W. H., Cameron, P. H., Doherty, J. J., 2nd, Posner, B. I. & Bergeron, J. J.
2048 Ligand-mediated autophosphorylation activity of the epidermal growth factor
2049 receptor during internalization. *J Cell Biol* **109**, 2751-2760,
2050 doi:10.1083/jcb.109.6.2751 (1989).

2051 255 Scita, G. & Di Fiore, P. P. The endocytic matrix. *Nature* **463**, 464-473,
2052 doi:10.1038/nature08910 (2010).

2053 256 Clevers, H. Modeling Development and Disease with Organoids. *Cell* **165**, 1586-
2054 1597, doi:10.1016/j.cell.2016.05.082 (2016).

2055 257 Mayle, K. M., Le, A. M. & Kamei, D. T. The intracellular trafficking pathway of
2056 transferrin. *Biochim Biophys Acta* **1820**, 264-281,
2057 doi:10.1016/j.bbagen.2011.09.009 (2012).

2058 258 Maurer, M. E. & Cooper, J. A. The adaptor protein Dab2 sorts LDL receptors into
2059 coated pits independently of AP-2 and ARH. *J Cell Sci* **119**, 4235-4246,
2060 doi:10.1242/jcs.03217 (2006).

2061 259 Mishra, S. K. *et al.* Disabled-2 exhibits the properties of a cargo-selective
2062 endocytic clathrin adaptor. *EMBO J* **21**, 4915-4926, doi:10.1093/emboj/cdf487
2063 (2002).

2064 260 Mishra, S. K., Watkins, S. C. & Traub, L. M. The autosomal recessive
2065 hypercholesterolemia (ARH) protein interfaces directly with the clathrin-coat
2066 machinery. *Proc Natl Acad Sci U S A* **99**, 16099-16104,
2067 doi:10.1073/pnas.252630799 (2002).

2068 261 Morris, S. M. & Cooper, J. A. Disabled-2 colocalizes with the LDLR in clathrin-
2069 coated pits and interacts with AP-2. *Traffic* **2**, 111-123, doi:10.1034/j.1600-
2070 0854.2001.020206.x (2001).

2071 262 Tao, W., Moore, R., Meng, Y., Smith, E. R. & Xu, X. X. Endocytic adaptors Arh and
2072 Dab2 control homeostasis of circulatory cholesterol. *J Lipid Res* **57**, 809-817,
2073 doi:10.1194/jlr.M063065 (2016).

2074 263 He, G. *et al.* ARH is a modular adaptor protein that interacts with the LDL
2075 receptor, clathrin, and AP-2. *J Biol Chem* **277**, 44044-44049,
2076 doi:10.1074/jbc.M208539200 (2002).

2077 264 Beglova, N. & Blacklow, S. C. The LDL receptor: how acid pulls the trigger. *Trends*
2078 *Biochem Sci* **30**, 309-317, doi:10.1016/j.tibs.2005.03.007 (2005).

2079 265 Renard, H. F. *et al.* Endophilin-A2 functions in membrane scission in clathrin-
2080 independent endocytosis. *Nature* **517**, 493-496, doi:10.1038/nature14064
2081 (2015).

2082 266 Simunovic, M. *et al.* Friction Mediates Scission of Tubular Membranes Scaffolded
2083 by BAR Proteins. *Cell* **170**, 172-184 e111, doi:10.1016/j.cell.2017.05.047 (2017).

2084 267 Galperin, E. & Sorkin, A. Endosomal targeting of MEK2 requires RAF, MEK kinase
2085 activity and clathrin-dependent endocytosis. *Traffic* **9**, 1776-1790,
2086 doi:10.1111/j.1600-0854.2008.00788.x (2008).

2087 268 Pinilla-Macua, I., Watkins, S. C. & Sorkin, A. Endocytosis separates EGF receptors
2088 from endogenous fluorescently labeled HRas and diminishes receptor signaling
2089 to MAP kinases in endosomes. *Proc Natl Acad Sci U S A* **113**, 2122-2127,
2090 doi:10.1073/pnas.1520301113 (2016).

2091 269 Sigismund, S. *et al.* Threshold-controlled ubiquitination of the EGFR directs
2092 receptor fate. *EMBO J* **32**, 2140-2157, doi:10.1038/emboj.2013.149 (2013).

2093 270 Rochman, Y., Spolski, R. & Leonard, W. J. New insights into the regulation of T
2094 cells by gamma(c) family cytokines. *Nat Rev Immunol* **9**, 480-490,
2095 doi:10.1038/nri2580 (2009).

2096 271 Basquin, C. *et al.* Membrane protrusion powers clathrin-independent
2097 endocytosis of interleukin-2 receptor. *EMBO J* **34**, 2147-2161,
2098 doi:10.15252/embj.201490788 (2015).

2099 272 Grassart, A., Dujeancourt, A., Lazarow, P. B., Dautry-Varsat, A. & Sauvonnet, N.
2100 Clathrin-independent endocytosis used by the IL-2 receptor is regulated by Rac1,
2101 Pak1 and Pak2. *EMBO Rep* **9**, 356-362, doi:10.1038/embor.2008.28 (2008).

2102 273 Sauvonnet, N., Dujeancourt, A. & Dautry-Varsat, A. Cortactin and dynamin are
2103 required for the clathrin-independent endocytosis of gammac cytokine receptor.
2104 *J Cell Biol* **168**, 155-163, doi:10.1083/jcb.200406174 (2005).

2105 274 Blasky, A. J., Mangan, A. & Prekeris, R. Polarized protein transport and lumen
2106 formation during epithelial tissue morphogenesis. *Annu Rev Cell Dev Biol* **31**, 575-
2107 591, doi:10.1146/annurev-cellbio-100814-125323 (2015).

2108 275 Winter, J. F. *et al.* Caenorhabditis elegans screen reveals role of PAR-5 in RAB-11-
2109 recycling endosome positioning and apicobasal cell polarity. *Nat Cell Biol* **14**, 666-
2110 676, doi:10.1038/ncb2508 (2012).

2111 276 Apodaca, G., Gallo, L. I. & Bryant, D. M. Role of membrane traffic in the
2112 generation of epithelial cell asymmetry. *Nat Cell Biol* **14**, 1235-1243,
2113 doi:10.1038/ncb2635 (2012).

2114 277 Henry, L. & Sheff, D. R. Rab8 regulates basolateral secretory, but not recycling,
2115 traffic at the recycling endosome. *Mol Biol Cell* **19**, 2059-2068,
2116 doi:10.1091/mbc.E07-09-0902 (2008).

2117 278 Babbey, C. M. *et al.* Rab10 regulates membrane transport through early
2118 endosomes of polarized Madin-Darby canine kidney cells. *Mol Biol Cell* **17**, 3156-
2119 3175, doi:10.1091/mbc.e05-08-0799 (2006).

2120 279 Lock, J. G. & Stow, J. L. Rab11 in recycling endosomes regulates the sorting and
2121 basolateral transport of E-cadherin. *Mol Biol Cell* **16**, 1744-1755,
2122 doi:10.1091/mbc.e04-10-0867 (2005).

2123 280 Duman, J. G., Tyagarajan, K., Kolsi, M. S., Moore, H. P. & Forte, J. G. Expression of
2124 rab11a N124I in gastric parietal cells inhibits stimulatory recruitment of the H+
2125 K+-ATPase. *Am J Physiol* **277**, C361-372, doi:10.1152/ajpcell.1999.277.3.C361
2126 (1999).

2127 281 Li, D., Mangan, A., Cicchini, L., Margolis, B. & Prekeris, R. FIP5 phosphorylation
2128 during mitosis regulates apical trafficking and lumenogenesis. *EMBO Rep* **15**,
2129 428-437, doi:10.1002/embr.201338128 (2014).

2130 282 Roland, J. T. *et al.* Rab GTPase-Myo5B complexes control membrane recycling
2131 and epithelial polarization. *Proc Natl Acad Sci U S A* **108**, 2789-2794,
2132 doi:10.1073/pnas.1010754108 (2011).

2133 283 Mendoza, M. C. *et al.* ERK-MAPK drives lamellipodia protrusion by activating the
2134 WAVE2 regulatory complex. *Mol Cell* **41**, 661-671,
2135 doi:10.1016/j.molcel.2011.02.031 (2011).

2136 284 Mendoza, M. C., Vilela, M., Juarez, J. E., Blenis, J. & Danuser, G. ERK reinforces
2137 actin polymerization to power persistent edge protrusion during motility. *Sci*
2138 *Signal* **8**, ra47, doi:10.1126/scisignal.aaa8859 (2015).

2139 285 Farooqui, R. & Fenteany, G. Multiple rows of cells behind an epithelial wound
2140 edge extend cryptic lamellipodia to collectively drive cell-sheet movement. *J Cell*
2141 *Sci* **118**, 51-63, doi:10.1242/jcs.01577 (2005).

2142 286 Giavazzi, F. *et al.* Flocking transitions in confluent tissues. *Soft Matter* **14**, 3471-
2143 3477, doi:10.1039/c8sm00126j (2018).

2144 287 Giavazzi, F. *et al.* Giant fluctuations and structural effects in a flocking
2145 epithelium. *Journal of Physics D: Applied Physics* **50**, 384003 (2017).

2146 288 Boucrot, E. & Kirchhausen, T. Endosomal recycling controls plasma membrane
2147 area during mitosis. *Proc Natl Acad Sci U S A* **104**, 7939-7944,
2148 doi:10.1073/pnas.0702511104 (2007).

2149 289 Tacheva-Grigorova, S. K., Santos, A. J., Boucrot, E. & Kirchhausen, T. Clathrin-
2150 mediated endocytosis persists during unperturbed mitosis. *Cell Rep* **4**, 659-668,
2151 doi:10.1016/j.celrep.2013.07.017 (2013).

2152 290 Aguet, F. *et al.* Membrane dynamics of dividing cells imaged by lattice light-sheet
2153 microscopy. *Mol Biol Cell* **27**, 3418-3435, doi:10.1091/mbc.E16-03-0164 (2016).

2154 291 Dix, C. L. *et al.* The Role of Mitotic Cell-Substrate Adhesion Re-modeling in
2155 Animal Cell Division. *Dev Cell* **45**, 132-145 e133,
2156 doi:10.1016/j.devcel.2018.03.009 (2018).

2157 292 Jones, M. C., Askari, J. A., Humphries, J. D. & Humphries, M. J. Cell adhesion is
2158 regulated by CDK1 during the cell cycle. *J Cell Biol* **217**, 3203-3218,
2159 doi:10.1083/jcb.201802088 (2018).

2160 293 Lock, J. G. *et al.* Reticular adhesions are a distinct class of cell-matrix adhesions
2161 that mediate attachment during mitosis. *Nat Cell Biol* **20**, 1290-1302,
2162 doi:10.1038/s41556-018-0220-2 (2018).

2163 **Mitotic matrix adhesion sites, termed ‘reticular adhesions’, are characterized in**
2164 **this study, showing that they are morphologically, dynamically and molecularly**
2165 **distinct from classical focal adhesions, being enriched in components of the**
2166 **clathrin machinery.**

2167 294 Zaidel-Bar, R. Atypical matrix adhesions guide cell division. *Nat Cell Biol* **20**, 1233-
2168 1235, doi:10.1038/s41556-018-0226-9 (2018).

2169 295 Elkhatib, N. *et al.* Tubular clathrin/AP-2 lattices pinch collagen fibers to support
2170 3D cell migration. *Science* **356**, doi:10.1126/science.aal4713 (2017).