Endocytosis in proliferating, quiescent, and terminally-differentiated cells

Claudia Hinze[1] and Emmanuel Boucrot[1,2,*]

[1] Institute of Structural and Molecular Biology, Division of Biosciences, University College London, London WC1E 6BT, UK
[2] Institute of Structural and Molecular Biology, Department of Biological Sciences, Birkbeck College, London WC1E 7HX, UK
*Correspondence: e.boucrot@ucl.ac.uk

Abstract

Endocytosis mediates nutrient uptake, receptor internalization and the regulation of cell signaling. It is also hijacked by many bacteria, viruses and toxins to mediate their cellular entry. Several endocytic routes exist in parallel, fulfilling different functions. Most studies on endocytosis have used transformed cells in culture. However, as the majority of cells in an adult body have exited the cell cycle, our understanding is biased towards proliferating cells. Here, we review the evidence of endocytosis not only in dividing, but also in quiescent, senescent or terminally-differentiated cells. During mitosis, residual endocytosis is dedicated to the internalization of caveolae and specific receptors. In non-dividing cells, Clathrin-mediated endocytosis (CME) is active, but alternative processes, such as caveolae, macropinocytosis and Clathrin-independent routes, vary widely depending on cell types and functions. Endocytosis supports the quiescent state by either up-regulating cell-cycle arrest pathways or down-regulating mitogen-induced signaling, thereby inhibiting cell proliferation. Endocytosis in terminally differentiated cells, such as skeletal muscles, adipocytes, kidney podocytes or neurons, supports tissue-specific functions. Finally, uptake is down-regulated in senescent cells, making them insensitive to proliferative stimuli by growth factors. Future studies should reveal the molecular basis for the differences in activities between the different cell states.

Introduction

Endocytosis is a ubiquitous cellular process essential for growth and survival. Extracellular macromolecules cannot be transported across the plasma membrane and must bind instead to cell surface transmembrane receptors and be internalized by endocytosis. Several parallel endocytic pathways (**Box 1**) mediate the uptake of nutrients and control cell surface receptor levels, plasma membrane turnover and cellular signaling and are required for cell spreading, polarization and migration (Barbieri et al., 2016). Because of their importance and evolutionary conservation, endocytic pathways are hijacked by many pathogens (Gruenberg and Van Der Goot, 2006). Furthermore, mutations resulting in mis-regulated endocytosis cause diseases, such as cancer, neurodegeneration, atherosclerosis and lysosomal storage diseases (Doherty and McMahon, 2009).

During endocytosis, the folding of the plasma membrane generates membrane invaginations of various sizes and shapes, which contain the cargo to be internalized. Nascent vesicles are subsequently

detached from the plasma membrane and traffic to their intracellular destinations. To date, there is evidence of three fundamental mechanisms generating endocytic carriers: (i) binding of cargo and localized membrane bending by cytosolic adaptor proteins; (ii) membrane bending induced by clustering of extracellular lipid or cargo (the so-called glycolipid-lectin [GL-Lect] hypothesis (reviewed in Johannes et al., 2016); and (iii) acute signal-induced membrane protrusions pushing outward of the cell and folding back onto themselves (reviewed in Ferreira and Boucrot, 2018). The multiplicity of endocytic pathways is consistent with the myriad of cellular processes they serve. Throughout the cell cycle, cells grow and need nutrients to synthesize proteins, DNA and lipids, undergo membrane remodeling during mitosis, or leave the cell cycle and stop dividing to perform specialized functions (**Box 2**). There are about 200 different cell types in an adult human body, each with specific needs linked to their physiological roles (Bianconi et al., 2013). Signaling mechanisms underlie biological functions and many are regulated by endocytosis, which connects the cell with its environment. In this review, we survey the evidence of endocytosis in dividing, and also non-dividing, cells such as quiescent, terminally differentiated and senescent cells. CME was reported to be active in all cellular states, albeit at different levels of efficacy, and to mediate the uptake of different cargoes. The study of Clathrin-independent pathways in non-proliferating cells has been lagging that of CME and thus it is not clear whether they are functioning in every cell state.

Endocytosis in dividing cells

The vast majority of our understanding of endocytosis comes from studies using proliferating cell lines. During exponential growth *in vitro*, cells continuously progress through the cell cycle and divide every 15 to 30 hours, depending on cell type. Analyses have been almost exclusively focused on cells during interphase (which constitutes over 98% of proliferating cultures; see also **Box 2**). Thus, it is widely assumed that endocytosis is similar during G1, S and G2, even though there is evidence of differences in uptake for some cargoes, depending on the cellular context (Snijder et al., 2009). For instance, cholera and Shiga toxins only enter cells during G1 and G2, respectively**.** This is because their cellular receptors, the glycosphingolipids monosialotetrahexosylganglioside (GM1) and globotriaosylceramide (Gb3), are only expressed at sufficiently high levels during these cell cycle phases (Majoul et al., 2002). Moreover, although CME is constitutively active (Bitsikas et al., 2014) at any given time, the activity of other pathways, such as macropinocytosis, Clathrin-independent carrier/GPI-anchored proteins-enriched carriers (CLIC/GEEC) or fast endophilin-ediated endocytosis (FEME) (**Box 1**), varies depending on the cellular state. Indeed, cell migration, cell-surface receptor activation and intracellular signaling and changes in membrane tension all stimulate Clathrin-independent endocytosis (CIE) (Lundmark et al., 2008; Boucrot et al., 2015; Holst et al., 2017).

The level of endocytosis during mitosis has been a topic of contention. The premise was that most cellular processes, other than microtubule spindle and cortical actin-driven cell rounding, cease during cell division. Indeed, transcription, translation and several other cellular functions slow down considerably during mitosis (Conrad, 1963; Fan and Penman, 1970; Gottesfeld and Forbes, 1997; Orthwein et al.,

2014). Some membrane flows also decrease during mitosis; for example, the Golgi apparatus disassembles, thus blocking protein secretion (Lucocq and Warren, 1987; Wei and Seemann, 2009), and endosomal recycling is strongly decreased, particularly during metaphase (Boucrot and Kirchhausen, 2007; Sager et al., 1984; Tacheva-Grigorova et al., 2013; Warren et al., 1984).

Endocytosis of typical CME cargoes, such as transferrin (Tf) or low density lipoprotein (LDL), is strongly reduced during mitosis (Pypaert et al., 1987; Fielding et al., 2012; Boucrot and Kirchhausen, 2007; Tacheva-Grigorova et al., 2013). The proposed mechanism for the inhibition of CME is the unavailability of actin to overcome the elevated plasma membrane tension in mitotic cells as free G-actin is recruited into cortical actin (Kaur et al., 2014). However, G-actin is unlikely to be rate limiting, as robust actin polymerization can be triggered in mitotic cells (Moulding et al., 2007; Santos et al., 2013). Instead, the strong reduction of Tf uptake is due to the low abundance of its receptor TfR at the cell surface of mitotic cells (**Fig. 1A**), as it becomes trapped inside cells when endosomal recycling shuts down (Sager et al., 1984; Warren et al., 1984; Boucrot and Kirchhausen, 2007; Tacheva-Grigorova et al., 2013). Clathrin-coated pits and vesicles continue to form during mitosis with the same characteristics (lifetimes and maximum intensities reached by the core adaptor AP2), albeit at lower rate during metaphase (Tacheva-Grigorova et al., 2013; Aguet et al., 2016) (**Fig. 1A**). This is consistent with the decrease in plasma membrane area during that phase of mitosis (Boucrot and Kirchhausen, 2007; Aguet et al., 2016). However, such endocytic activity is only preserved in unperturbed cells, whereas CME is inhibited upon chemical synchronization that is commonly used to stall cells in metaphase, *e.g.* use of nocodazole, RO-3306 or S-Trityl-L-cysteine (Fielding et al., 2012; Tacheva-Grigorova et al., 2013; Aguet et al., 2016). In such cells, Tf uptake is inhibited despite high levels of TfR at the surface (**Fig. 1B**), because no Clathrin-coated pits are forming anymore (Tacheva-Grigorova et al., 2013). The usefulness of such residual CME has been questioned, but there is now several direct evidence of uptake of endogenous cargoes into dividing cells both *in vitro* and *in vivo* (**Fig. 1D-F**) (Bökel et al., 2006; Coumailleau et al., 2009; Devenport et al., 2011; Cota and Davidson, 2015; Heck and Devenport, 2017).

In the absence of its typical cargoes, residual CME during mitosis is dedicated to the internalization of specific receptors, which are TGF-β receptor-type morphogen decapentaplegic (Dpp), fibroblast growth factor receptor (FGFR), Notch receptor, and the planar cell polarity (PCP) complex components Celsr1, Frizzled 6 and Vangl2 (**Fig. 1D-F**) (Bökel et al., 2006; Coumailleau et al., 2009; Devenport et al., 2011; Cota and Davidson, 2015; Heck and Devenport, 2017). Interestingly, some CIE events also occur during mitosis, such as the Clathrin-independent uptake of epidermal growth factor receptor (EGFR) (Liu et al., 2011) (**Fig. 1G**), and even the very efficient entry of *Salmonella* into mitotic cells (**Fig. 1C**), in a actin-driven process akin to macropinocytosis (Santos et al., 2013).

A function for the dedicated endocytosis of receptors into endosomes during mitosis is to mediate their equal or asymmetrical partitioning between the two daughter cells (**Fig. 1I**). Both Dpp and the PCP complex are polarized at the surface before cell division but yet need to be inherited equally to sustain tissue polarity (**Fig. 1I**). Indeed, blocking their uptake during mitosis *in vivo* induced a defective partitioning

between daughter cells, thereby severely compromising tissue polarity (Bökel et al., 2006; Heck and Devenport, 2017). At the reverse, the biased partitioning of Notch and Delta as well as of FGFR during mitosis of stem cells is mediating the asymmetrical fate of the daughter cells during organ development and polarization: the cell keeping the receptors having a different fate than the other one (Cota and Davidson, 2015; Coumailleau et al., 2009; Derivery et al., 2015). This is mediated by endocytosis of the receptors during cell division, followed by active targeting of the endosomes containing them (**Fig. 1I**). Later, during cytokinesis, endocytosis is localized at the forming cleavage furrow and supports the membrane fluxes that are required for changes in membrane shape abscission separating the two daughters cells (reviewed in Frémont and Echard, 2018).

Caveolae are also actively internalized during cell division (Boucrot et al., 2011). They enter cells during the mitotic roundup until metaphase (**Fig. 1H**) and return to the cell surface after anaphase and during cytokinesis, perhaps to ensure equal inheritance between the two daughter cells (**Fig. 1I**). These fluxes mirror that of the receptors internalized during mitosis, which enter cells but fail to return to the cell surface because of the shut-down in endosomal recycling until the subsequent onset of anaphase (Boucrot and Kirchhausen, 2007; Tacheva-Grigorova et al., 2013). Interestingly, mitotic Polo-like kinase 1 (Plk1) was found to be critical for the uptake of the PCP receptor Celsr1 and its retention into endosomes, providing a rationale between mitosis progression and the regulation of membrane traffic (Shrestha et al., 2015). Thus, endocytosis in proliferating cells switches from mediating the uptake of a large number of cargoes during interphase to be dedicated to the internalization of specific receptors that must be redistributed equally or asymmetrically between the two daughter cells. This also illustrates that endocytosis varies depending on the cellular state, and thus, it is logical that non-dividing cells, such as quiescent, senescent or terminally-differentiated cells, have different endocytic needs and mechanisms than proliferating ones, as the following sections will review.

Endocytosis in quiescent cells

Cellular quiescence (also named ‘G0’ stage of the cell cycle) is the state in which cells are not dividing but retain the ability to resume proliferation upon stimulation (**Box 2**). Many cells in an adult body, including endothelial cells, mature hepatocytes and dormant tissue stem cells, reside in a quiescent state. They can re-enter the cell cycle upon external stimuli, such as injury or to maintain tissue homeostasis. Quiescent cells exhibit varying metabolic activity, but display reduced protein synthesis and cellular growth (Cho and Hwang, 2012; Lemons et al., 2010; Shapiro, 1981; Yusuf and Fruman, 2003) (**Box 2**). Endocytic mechanisms during this cellular state are still poorly understood, but evidence exists that endocytosis supports cell-type specific functions. Such functions include clearance of the blood from harmful substances (*e.g.* LDL in liver), uptake of nutrients, such as iron and cholesterol (which can be stored), formation of a primary cilium, cell polarization and control of cell-cell junctions (Goto et al., 2017; Lin et al., 2015; Nunez et al., 1996; Zanoni et al., 2018). Trans-endocytosis (also called transcytosis) is also a feature of some quiescent cells and mediates the transport of ligands and receptors across epithelial and

endothelial barriers (reviewed in Rodriguez-Boulan et al., 2005). Finally, endocytosis maintains the quiescent state by either down-regulating mitogen-induced signaling (Koo et al., 2012; Nakayama et al., 2013), or up-regulating cell-cycle arrest pathways (Pedersen et al., 2016). It also mediates the cellular uptake of extracellular proteins, which can then be degraded and the amino acids used to sustain survival during cell quiescence (Muranen et al., 2017).

Many quiescent cells develop a primary cilium, which senses the availability of extracellular nutrients and growth factors (reviewed in Goto et al., 2017). The ciliary pocket at the base of the cilium is a site of active endocytosis, characterized by an abundance of clathrin-coated pits and vesicles (Ghossoub et al., 2016; Molla-Herman et al., 2010). Endocytosis at the ciliary pocket controls ciliary Sonic Hedgehog (Shh) and TGF-β signaling (**Fig. 2A**), potentially supporting the non-proliferative state of quiescent cells (Pedersen et al., 2016). Most quiescent cells are part of tissues and form junctional cell-cell contacts on their basolateral membranes: adherens junctions (AJs) composed of cadherins, tight junctions (TJs) formed by claudins, occludins and ZO proteins, and gap junctions (GJs) comprising connexins (Radeva and Waschke, 2018). Endocytosis of endothelial (E)- and vascular endothelial (VE)-Cadherin is required for the formation and maintenance of mature AJs in quiescent epithelial and endothelial cells (**Fig. 2C)** (de Beco et al., 2009; Nanes et al., 2012).

Mechanistically, E-Cadherin uptake at AJs requires the endocytic proteins CIP4 and Dynamin, as well as local actin polymerization that is mediated by Cdc42, Arf6-, N-WASP and Arp2/3 (Druso et al., 2016; Georgiou et al., 2008; Leibfried et al., 2008; Palacios et al., 2002). The precise endocytic pathway is still unclear, but these are molecular factors that act both in CME and in FEME (Chan Wah Hak et al., 2018; Taylor et al., 2011). Furthermore, it is unclear whether the mechanism of uptake of free cadherins is similar to those clustered at AJs. Cadherins have a conserved binding motif for the core CME adaptor AP2 in their cytoplasmic domains, but it is obstructed upon binding to β-catenin and p120 catenin in AJs (Miyashita and Ozawa, 2007; Nanes et al., 2012). Thus, CME might mediate the uptake of free but not AJ-clustered Cadherins. Alternatively, the uptake might be independent of AP2, as is the case upon clustering of E-cadherin by the *Listeria* protein InlA that triggers the recruitment of the adaptor Dab2, followed by that of Clathrin and actin (Bonazzi et al., 2011; Veiga and Cossart, 2005; Veiga et al., 2007).

TJs form a diffusion barrier in quiescent endothelial and epithelial cells and are key to the impermeability of the blood brain barrier (Stamatovic et al., 2017). Upon stimuli, such as growth factor addition or Calcium decrease, the removal of claudins, occludin and ZO-1 from TJs is mediated by CME (**Fig. 2B)** (Cong et al., 2015; Ikari et al., 2011; Yamaki et al., 2014). However, the mechanism for the constitutive uptake of TJ components, while maintaining their barrier function, is still unclear (Dukes et al., 2011; Stamatovic et al., 2017). It has recently been proposed that TJ remodeling is mediated by so-called 'cross-over' endocytosis, the removal of TJs from one cell into its neighbor within a double-membrane vesicle (Gehne et al., 2017). Although molecular details are still missing, this process appears to be constitutive and can internalize entire TJs and not only specific claudins.

Finally, GJs form intercellular connections, which allow various small molecules, ions and electrical impulses to pass directly between neighboring quiescent cells. Growth factor signals, such as EGF and VEGF, prime quiescent cell layers for their disassembly and cell cycle re-entry by stimulating junction disassembly (Fong et al., 2014; Nimlamool et al., 2015). The concomitant PKC- and MAPK-induced phosphorylation of Connexin 43 licenses it for cellular uptake through CME, thereby disassembling GJs (**Fig. 2D**).

Endocytosis is used in quiescent cells to modulate the availability of many growth factor and cytokine receptors at their surface, either through their downregulation or maintenance. For instance, although VEGF internalization and concentration into endosomes is required for signaling and stimulates angiogenesis, its uptake into mature blood vessels is reduced (Nakayama et al., 2013). This decrease is mediated by the phosphorylation of the Clathrin adaptor Dab2 by atypical PKC, which blocks the binding of Dab2 to VEGFR and thereby inhibits its endocytosis (Nakayama et al., 2013). Conversely, the continuous cell-surface removal of several receptors by endocytosis is required to prevent cell cycle re-entry and proliferation of several types of quiescent cells. CME and lysosomal degradation of the tyrosine-kinase receptor Kit maintains the non-proliferative state of mast cells (Cruse et al., 2015). In intestinal crypts, $Lgr5^+$ stem cells remain quiescent by escaping Wnt-mediated catenin signaling through the active removal of the Frizzled receptor from the cell surface (Koo et al., 2012). There, the stem cell-specific E3 ligase RNF43 ubiquitinates Frizzled and induces its endocytosis and subsequent degradation in lysosomes (Koo et al., 2012). Consistently, blocking CME of the intestinal crypt stem cell marker Lgr5 diminishes cell fitness, and the broader inhibition of endocytosis by blocking Dynamin in intestinal stem cells induces their hyper-proliferation and leads to a severe defect in epithelial homeostasis (Nagy et al., 2016; Snyder et al., 2017).

A third function of endocytosis might be to support the survival of quiescent cells. Lack of growth factor stimulation reduces mTORC1 activity in G0 cells (Gan and DePinho, 2009) (**Box 2**). Increased cell-surface expression of β4-integrin in quiescent cells mediates the cellular uptake of its extracellular matrix (ECM) ligands, the Laminins (Muranen et al., 2017). The lysosomal degradation of Laminins produces free amino acids, thereby restoring a basal mTORC1 activity and promoting survival (Muranen et al., 2017). The precise mechanism of β4-integrin uptake is, however, unclear. β4-integrin forms heterodimers with α6 chains, which contain a cytoplasmic Yxxφ motif that can interact with AP2 (De Franceschi et al., 2016), suggesting that CME might mediate such uptake (**Fig. 2E).** To conclude, endocytosis is required for quiescent cells to perform specific cellular funtions as well as modulating their cell cycle signaling and survival. However, the precise pathways and mechanisms are still porrly understood.

Endocytosis in terminally differentiated cells

Terminally differentiated cells have irreversibly exited the cell cycle and cannot resume proliferation (**Box 2**). Mature neurons, skeletal muscles, kidney podocytes, adipocytes or intestine enterocytes are highly specialized and perform tissue-specific functions (Guo et al., 2009; Herrup and Busser, 1995; Lasagni et

al., 2013; Latella et al., 2001). Therefore, their endocytic activities differ widely depending on the exact cell types and functions performed, as outlined below.

Endocytosis at neuronal synapses is required following neurotransmitter release for the rapid recycling of synaptic proteins from the cell surface. Ultrafast endocytosis, which is Clathrin-independent and Endophilin- and Dynamin-dependent, and perhaps reminiscent of FEME (see **Box1**), retrieves membranes and proteins from the synaptic cleft within milliseconds (Gan and Watanabe, 2018; Watanabe et al., 2018). CME mediates synaptic vesicle recycling as well, but does so away from the active synaptic zone and with slower kinetics (Gan and Watanabe, 2018). A third form of uptake, activity-dependent bulk endocytosis (ADBE), operates in response to sustained and elevated neuron stimulation and shares similarities with macropinocytosis (Cousin, 2017). Endocytosis at the synapse has been intensely studied and summarized in recent reviews (Cousin, 2017; Gan and Watanabe, 2018; Maritzen and Haucke, 2017).

Endocytosis also occurs at the postsynaptic membrane, reducing surface-receptor levels after long patterned stimuli, a mechanism known as long-term depression (LTD). The most common LTD mechanism involves the downregulation of postsynaptic heterotetrameric α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) from the surface of glutaminergic synapses. Constitutive endocytosis of AMPAR at the postsynaptic membrane is believed to be Clathrin-independent (Fujii et al., 2017). However, constitutive CME of the receptor, as well as other cargoes, were reported to occur there, as well at dendrites and in the soma (Rosendale et al., 2017). Upon LTD-inducing stimulation, AMPAR is sorted into Clathrin-coated pits and efficiently removed from the plasma membrane, thereby reducing the sensitivity of neurons to neurotransmitters (Lee et al., 2002; Rosendale et al., 2017). During axon growth and before synapse formation, macropinocytosis, CME and an Endophilin-dependent pathway (akin to FEME) are required to modulate attractive and repulsive receptors (Chen and Tai, 2017; Tojima and Kamiguchi, 2015; Chang et al., 2017)). Finally, macropinocytosis mediates neuron-to-neuron transmission of protein aggregates, perhaps also supporting the spread of amyloids during neurodegenerative diseases (Yerbury, 2016).

Skeletal muscle fibers form large flat AP2 and Clathrin lattices (Vassilopoulos et al., 2014; Liu et al., 2018), which, together with actin and alpha-actinin, control sarcomere maintenance, but are also endocytically active. Cardiomyocytes display active endocytosis of transferrin and integrins by CME, and of dextran by macropinocytosis (Ottesen et al., 2015; Soeiro et al., 2002; Swildens et al., 2010). They also actively internalize β1-adrenergic receptor (Morisco et al., 2008), which is mostly entering cells through FEME in proliferating cells (Boucrot et al., 2015). Interestingly, Dab2 may not be involved in cardiomyocyte gap junction remodeling, contrary to its role in quiescent epithelial and endothelial cells (Waxse et al., 2017), suggesting differences in the underlying mechanisms.

Another type of terminally differentiated cells with reported endocytic activity are adipocytes. CME is active in adipocytes and mediates the uptake of typical CME cargoes such as transferrin (Kao et al., 1998), as well as the internalization of the key glucose transporter GLUT4 upon insulin stimulation (Blot

and McGraw, 2006; Shigematsu et al., 2003). Endocytosis of GLUT4 in resting adipocytes occurs primarily through a clathrin-independent pathway, perhaps caveolae, which, however, is inhibited following insulin stimulation, thereby allowing CME to take over the transporter uptake. Insulin-induced GLUT4 internalization in muscle cells differs from adipocytes in that it is insensitive to the disruption of caveolae endocytosis, but is instead completely abrogated upon Dynamin inhibition (Antonescu et al., 2008). Both adipocytes and cardiomyocytes exhibit striking amounts of caveolae (Thorn et al., 2003), but it is not clear how many of them mediate the actual uptake of cargoes. Mechanoprotection and provision of membrane reservoirs might be the prevailing functions of caveolae in these cells, as both adipocytes and myoblasts undergo dramatic changes in size and shape upon lipid storage, or contraction and hypertrophy, respectively (Kozera et al., 2009; Huang et al., 2013; Lo et al., 2015; Briand et al., 2014).

Podocyte epithelia develop specialized foot processes that are connected by the slit diaphragm, forming a size-selective filtration barrier (reviewed in Inoue and Ishibe, 2015). Endocytic processes (primarily CME) and actin remodeling play a major role in the maintenance of the filtration barrier and the uptake of integrins and lipoproteins (reviewed in Inoue and Ishibe, 2015). The formation of podocytes is dependent on the CME and FEME proteins Dynamin, Synaptojanin and Endophilin (Soda et al., 2012). It has been shown that the integrity of the slit diaphragm is maintained by the interaction of the receptor Nephrin with Podocin and its endocytosis via Clathrin-independent endocytosis (Qin et al., 2009). The BAR domain protein Pacsin-2 has been shown to play a role in Nephrin uptake, but the molecular details of the endocytic pathway remain unclear (Dumont et al., 2017).

Recent work measuring CME in isogenic cells derived from gene-edited human embryonic stem cells (hESCs) revealed striking differences in endocytic activity and mechanisms upon differentiation (Dambournet et al., 2018; Schöneberg et al., 2018). Intestinal epithelial cells differentiated from hESCs and grown into organoids had uniform CME dynamics both at their apical, lateral and basal membranes (Schöneberg et al., 2018). Moreover, both hESCs and derived neuronal progenitor cells (NPCs) had rapid (~45 sec) and productive formation of Clathrin-coated vesicles (Dambournet et al., 2018). However, cells differentiated into fibroblasts showed slower (~75 sec) and less productive CME. This was correlated with a doubling in AP2 levels upon differentiation, which, once it had been corrected back to levels close to that of the parental hESCs, restored efficient and rapid CME (Dambournet et al., 2018). In addition, unlike in hESCs and NPCs, CME in fibroblasts did not require the actin cytoskeleton (Dambournet et al., 2018). Finally, inhibition of phosphoinositide 3-kinase (PI3K), while having no effect in hESCs, improved the productivity of CME in fibroblasts, but decreaed it in NPCs (Dambournet et al., 2018). Thus, these experiments show convincingly that the molecular needs for CME are distinct depending on the cell types and adapt upon differentiation.

Endocytosis in senescent cells

Cellular senescence is the state in which normal, non-transformed, cells cease to replicate permanently, following telomere shortening beyond a critical length, or irreversible DNA damage (Muñoz-Espín and

Serrano, 2014) (**Box 2**). High levels of β-galactosidase and $p16^{Ink4A}$ are typically used to identify senescent cells (Sharpless and Sherr, 2015). Only cancer cells escape senescence, as mutations in the machinery mediating telomere shortening and DNA damage checkpoints, in particular p53, are hallmarks of oncogenic transformation (Hanahan and Weinberg, 2011). Non-transformed cells become senescent upon aging and might constitute the majority of cells in an old organism (**Box 2**). As many cellular processes are altered during senescence, it is not surprising that endocytosis is perturbed as well. Senescent fibroblasts retain normal levels of growth factor receptors and associated signaling proteins, but do not respond to proliferative stimuli by growth factors such as EGF, even at very high doses (Park et al., 2000). Thus, they differ from quiescent cells in that they cannot re-enter the cell cycle, sustain high mTORC1 activity, and are not able to generate functional primary cilia (Carroll et al., 2017; reviewed in Terzi et al., 2016).

The literature measuring endocytosis in naturally occurring senescent cells instead of acutely damaged cells (*e.g.* peroxide- or high UV doses-induced) is still very limited. However, the hypo-responsiveness of senescent cells to growth factors may be explained by the concomitant (i) up-regulation of Caveolin-1 and -2 levels (Park et al., 2000); (ii) the paradoxical absence of functional caveolae, which impairs EGFR dimerization and activation (Ikonen and Parton, 2000; Wheaton et al., 2001); and (iii) the down-regulation of the Clathrin adaptor Amphiphysin, which could account for the decreased CME (Park et al., 2001). Reduction of Caveolin-1 and overexpression of Amphiphysin were proposed to be sufficient to restore the responsiveness of senescent cells to growth factors (Park et al., 2000 and Cho et al., 2003).

In addition, cells with elevated senescent-specific splice variants of the transcriptional regulator ING1 overexpress the Clathrin adaptor scaffold Intersectin-2 (Rajarajacholan et al., 2013). Over-representation of Intersectin-2 disrupts the stoichiometry required for Clathrin-coated pit formation, resulting in impaired endocytosis and activation of the $p16^{INK4a}$ senescence signaling axis (Rajarajacholan et al., 2013). However, reduced CME is unlikely to be sufficient to induce senescence, as the knock down of AP2 causes growth arrest, but does not recapitulate the senescent phenotype (Olszewski et al., 2014). Thus, the responses to irreversible DNA damage or critical telomere shortening might induce some adaptations in endocytosis, but the molecular details are yet to be fully elucidated.

Conclusions and future perspectives

The various cell types in an organism reside in different proliferative states to serve distinct physiological functions and it is therefore only logical that they have different endocytic needs. Our molecular understanding of endocytosis in non-proliferating cells is lagging behind that of dividing cells, so it is still too early to conclude whether the mechanisms used by each pathway differ in these different scenarios. However, current evidence supports the notion that CME is broadly active in dividing, quiescent and terminally differentiated cells, but perturbed in senescent cells. Yet, the cargoes internalised by CME vary depending on the specific cell cycle state. The activity of clathrin-independent pathways including

macropinocytosis also varies in different cell states and is perhaps linked to the specialized functions performed by either quiescent or terminally differentiated cells.

Furthermore, it is important to remember that most of our current knowledge of endocytic mechanisms is derived from studies of proliferating cells, and the mechanisms prevailing in non-dividing cells might be quite different, especially because *in vitro* cell lines are all transformed cell lines, with the exception of hTERT-immortalized diploid cell lines (Bodnar, 1998). Indeed, cancer cells bear many mutations and often have different endocytosis activity compared to their non-tumorous counterparts (Elkin et al., 2015). Some transformed cells have elevated and adaptive CME (Chen et al., 2017), which may support cancer cell survival and metastasis (reviewed in Schmid, 2017). The frequent G12V activating mutation of K-Ras reduces CME and to some extent clathrin-independent uptake, but induces constitutive macropinocytosis (Commisso et al., 2013; Elkin et al., 2015). Thus, it is possible that many studies in the literature have been reporting endocytic mechanisms that might be more closely describing tumour rather than normal cells. Further characterization of proliferating non-cancer cells might thus help to us to gain a better understanding of endocytosis and serve as a useful reference for quiescent, terminally differentiated or senescent cells.

Funding

C.H. was supported by a studentship from the British Heart Foundation (FS/14/20/30681). E.B. is a Lister Institute Research Fellow.

References

Adhikari, D., Zheng, W., Shen, Y., Gorre, N., Hämäläinen, T., Cooney, A. J., Huhtaniemi, I., Lan, Z.-J. and Liu, K. (2010). Tsc/mTORC1 signaling in oocytes governs the quiescence and activation of primordial follicles. *Hum. Mol. Genet.* **19**, 397–410.

Aguet, F., Upadhyayula, S., Gaudin, R., Chou, Y., Cocucci, E., He, K., Chen, B.-C., Mosaliganti, K., Pasham, M., Skillern, W., et al. (2016). Membrane dynamics of dividing cells imaged by lattice light-sheet microscopy. *Mol. Biol. Cell* **27**, 3418–3435.

Antonescu, C. N., Díaz, M., Femia, G., Planas, J. V. and Klip, A. (2008). Clathrin-Dependent and independent endocytosis of glucose transporter 4 (GLUT4) in myoblasts: Regulation by mitochondrial uncoupling. *Traffic* **9**, 1173–1190.

Antonny, B., Burd, C., De Camilli, P., Chen, E., Daumke, O., Faelber, K., Ford, M., Frolov, V. A., Frost, A., Hinshaw, J. E., et al. (2016). Membrane fission by dynamin: what we know and what we need to know. *EMBO J.* **35**, 2270–2284.

Barbieri, E., Di Fiore, P. P. and Sigismund, S. (2016). Endocytic control of signaling at the plasma membrane. *Curr. Opin. Cell Biol.* **39**, 21–27.

Bianconi, E., Piovesan, A., Facchin, F., Beraudi, A., Casadei, R., Frabetti, F., Vitale, L., Pelleri, M. C., Tassani, S., Piva, F., et al. (2013). An estimation of the number of cells in the human body. *Ann. Hum. Biol.* **40**, 463–471.

Bitsikas, V., Corrêa, I. R. and Nichols, B. J. (2014). Clathrin-independent pathways do not contribute significantly to endocytic flux. *Elife* **3**, e03970.

Blot, V. and McGraw, T. E. (2006). GLUT4 is internalized by a cholesterol-dependent nystatin-sensitive mechanism inhibited by insulin. *EMBO J.* **25**, 5648–5658.

Bodnar, a. G. (1998). Extension of Life-Span by Introduction of Telomerase into Normal Human Cells. *Science (80-.).* **279**, 349–352.

Bökel, C., Schwabedissen, A., Entchev, E., Renaud, O. and González-Gaitán, M. (2006). Sara endosomes and the maintenance of Dpp signaling levels across mitosis. *Science (80-.).* **314**, 1135–1139.

Bonazzi, M., Vasudevan, L., Mallet, A., Sachse, M., Sartori, A., Prevost, M. C., Roberts, A., Taner, S. B., Wilbur, J. D., Brodsky, F. M., et al. (2011). Clathrin phosphorylation is required for actin recruitment at sites of bacterial adhesion and internalization. *J. Cell Biol.* **195**, 525–536.

Boucrot, E. and Kirchhausen, T. (2007). Endosomal recycling controls plasma membrane area during mitosis. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 7939–44.

Boucrot, E., Howes, M. T., Kirchhausen, T. and Parton, R. G. (2011). Redistribution of caveolae during mitosis. *J. Cell Sci.* **124**, 1965–1972.

Boucrot, E., Ferreira, A. P. A., Almeida-Souza, L., Debard, S., Vallis, Y., Howard, G., Bertot, L., Sauvonnet, N. and McMahon, H. T. (2015). Endophilin marks and controls a clathrin-independent endocytic pathway. *Nature* **517**, 460–465.

Briand, N., Prado, C., Mabilleau, G., Lasnier, F., Le Lièpvre, X., Covington, J. D., Ravussin, E., Le Lay, S. and Dugail, I. (2014). Caveolin-1 expression and cavin stability regulate caveolae dynamics in adipocyte lipid store fluctuation. *Diabetes* **63**, 4032–4044.

Buttitta, L. A. and Edgar, B. A. (2007). Mechanisms controlling cell cycle exit upon terminal differentiation. *Curr. Opin. Cell Biol.* **19**, 697–704.

Cameron, I. L. and Greulich, R. C. (1963). Evidence for an essentially constant duration of DNA synthesis in renewing epithelia of the adult mouse. *J. Cell Biol.* **18**, 31–40.

Campisi, J. (2005). Senescent cells, tumor suppression, and organismal aging: Good citizens, bad neighbors. *Cell* **120**, 513–522.

Carroll, B., Nelson, G., Rabanal-Ruiz, Y., Kucheryavenko, O., Dunhill-Turner, N. A., Chesterman, C. C., Zahari, Q., Zhang, T., Conduit, S. E., Mitchell, C. A., et al. (2017). Persistent mTORC1 signaling in cell senescence results from defects in amino acid and growth factor sensing. *J. Cell Biol.* **216**, 1949–1957.

Chan Wah Hak, L., Khan, S., Meglio, I. Di, Law, A.-L., Lucken-Ardjomande Häsler, S., Quintaneiro, L. M., Ferreira, A. P. A., Krause, M., McMahon, H. T. and Boucrot, E. (2018). FBP17 and CIP4 recruit SHIP2 and lamellipodin to prime the plasma membrane for fast endophilinmediated endocytosis. *Nat. Cell Biol.* **In Press**,.

Chang, T. Y., Chen, C., Lee, M., Chang, Y. C., Lu, C. H., Lu, S. T., Wang, D. Y., Wang, A., Guo, C. L. and Cheng, P. L. (2017). Paxillin facilitates timely neurite initiation on soft-substrate environments by interacting with the endocytic machinery. *Elife* **6**, e31101.

Chen, Y. T. and Tai, C. Y. (2017). μ2-Dependent endocytosis of N-cadherin is regulated by β-catenin to facilitate neurite outgrowth. *Traffic* **18**, 287–303.

Chen, P. H., Bendris, N., Hsiao, Y. J., Reis, C. R., Mettlen, M., Chen, H. Y., Yu, S. L. and Schmid, S. L. (2017). Crosstalk between CLCb/Dyn1-Mediated Adaptive Clathrin-Mediated Endocytosis and Epidermal Growth Factor Receptor Signaling Increases Metastasis. *Dev. Cell* **40**, 278–288.e5.

Cheung, T. H. and Rando, T. A. (2013). Molecular regulation of stem cell quiescence. *Nat. Rev. Mol. Cell Biol.* **14**, 329–340.

Cho, S. and Hwang, E. S. (2012). Status of mTOR activity may phenotypically differentiate senescence and quiescence. *Mol. Cells* **33**, 597–604.

Cho, K. A., Ryu, S. J., Park, J. S., Jang, I. S., Ahn, J. S., Kim, K. T. and Park, S. C. (2003). Senescent phenotype can be reversed by reduction of caveolin status. *J. Biol. Chem.* **278**, 27789–27795.

Coller, H. a, Sang, L. and Roberts, J. M. (2006). A new description of cellular quiescence. *PLoS Biol.* **4**, e83.

Commisso, C., Davidson, S. M., Soydaner-Azeloglu, R. G., Parker, S. J., Kamphorst, J. J., Hackett, S., Grabocka, E., Nofal, M., Drebin, J. A., Thompson, C. B., et al. (2013). Macropinocytosis of protein is an amino acid supply route in Ras-transformed cells. *Nature* **497**, 633–7.

Cong, X., Zhang, Y., Li, J., Mei, M., Ding, C., Xiang, R.-L., Zhang, L.-W., Wang, Y., Wu, L.-L. and Yu, G.-Y. (2015). Claudin-4 is required for modulation of paracellular permeability by muscarinic acetylcholine receptor in epithelial cells. *J. Cell Sci.* **128**, 2271–2286.

Conrad, C. G. (1963). Protein synthesis and RNA synthesis during mitosis in animal cells. *J. Cell Biol.* **19**, 267–277.

Cota, C. D. and Davidson, B. (2015). Mitotic Membrane Turnover Coordinates Differential Induction of the Heart Progenitor Lineage. *Dev. Cell* **34**, 505–519.

Coumailleau, F., Fürthauer, M., Knoblich, J. A. and González-Gaitán, M. (2009). Directional Delta and Notch trafficking in Sara

endosomes during asymmetric cell division. *Nature* **458**, 1051–1055.
Cousin, M. A. (2017). Integration of Synaptic Vesicle Cargo Retrieval with Endocytosis at Central Nerve Terminals. *Front. Cell. Neurosci.* **11**, 234.
Cruse, G., Beaven, M. A., Music, S. C., Bradding, P., Gilfillan, A. M. and Metcalfe, D. D. (2015). The CD20 homologue MS4A4 directs trafficking of KIT toward clathrin-independent endocytosis pathways and thus regulates receptor signaling and recycling. *Mol. Biol. Cell* **26**, 1711–1727.
Dambournet, D., Sochacki, K. A., Cheng, A. T., Akamatsu, M., Taraska, J. W., Hockemeyer, D. and Drubin, D. G. (2018). Genome-edited human stem cells expressing fluorescently labeled endocytic markers allow quantitative analysis of clathrin-mediated endocytosis during differentiation. *J. Cell Biol.* **217**, jcb.201710084.
de Beco, S., Gueudry, C., Amblard, F. and Coscoy, S. (2009). Endocytosis is required for E-cadherin redistribution at mature adherens junctions. *Proc. Natl. Acad. Sci.* **106**, 7010–7015.
De Franceschi, N., Arjonen, A., Elkhatib, N., Denessiouk, K., Wrobel, A. G., Wilson, T. A., Pouwels, J., Montagnac, G., Owen, D. J. and Ivaska, J. (2016). Selective integrin endocytosis is driven by interactions between the integrin α-chain and AP2. *Nat. Struct. Mol. Biol.* **23**, 172–179.
Derivery, E., Seum, C., Daeden, A., Loubéry, S., Holtzer, L., Jülicher, F. and Gonzalez-Gaitan, M. (2015). Polarized endosome dynamics by spindle asymmetry during asymmetric cell division. *Nature* **528**, 280–285.
Devenport, D., Oristian, D., Heller, E. and Fuchs, E. (2011). Mitotic internalization of planar cell polarity proteins preserves tissue polarity. *Nat. Cell Biol.* **13**, 893–902.
Doherty, G. J. and McMahon, H. T. (2009). Mechanisms of endocytosis. *Annu. Rev. Biochem.* **78**, 857–902.
Druso, J. E., Endo, M., Joy Lin, M. C., Peng, X., Antonyak, M. A., Meller, S. and Cerione, R. A. (2016). An essential role for Cdc42 in the functioning of the adult mammary gland. *J. Biol. Chem.* **291**, 8886–8895.
Dukes, J. D., Fish, L., Richardson, J. D., Blaikley, E., Burns, S., Caunt, C. J., Chalmers, A. D. and Whitley, P. (2011). Functional ESCRT machinery is required for constitutive recycling of claudin-1 and maintenance of polarity in vertebrate epithelial cells. *Mol. Biol. Cell* **22**, 3192–3205.
Dumont, V., Tolvanen, T. A., Kuusela, S., Wang, H., Nyman, T. A., Lindfors, S., Tienari, J., Nisen, H., Suetsugu, S., Plomann, M., et al. (2017). PACSIN2 accelerates nephrin trafficking and is up-regulated in diabetic kidney disease. *FASEB J.* **31**, 3978–3990.
Elkin, S. R., Bendris, N., Reis, C. R., Zhou, Y., Xie, Y., Huffman, K. E., Minna, J. D. and Schmid, S. L. (2015). A systematic analysis reveals heterogeneous changes in the endocytic activities of cancer cells. *Cancer Res.* **75**, 4640–4650.
Fan, H. and Penman, S. (1970). Regulation of protein synthesis in mammalian cells. II. Inhibition of protein synthesis at the level of initiation during mitosis. *J. Mol. Biol.* **50**, 655–670.
Ferreira, A. P. A. and Boucrot, E. (2018). Mechanisms of Carrier Formation during Clathrin-Independent Endocytosis. *Trends Cell Biol.* **28**, 188–200.
Fielding, A. B., Willox, A. K., Okeke, E. and Royle, S. J. (2012). Clathrin-mediated endocytosis is inhibited during mitosis. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 6572–7.
Flannagan, R. S., Jaumouillé, V. and Grinstein, S. (2012). The Cell Biology of Phagocytosis. *Annu. Rev. Pathol. Mech. Dis.* **7**, 61–98.
Fong, J. T., Nimlamool, W. and Falk, M. M. (2014). EGF induces efficient Cx43 gap junction endocytosis in mouse embryonic stem cell colonies via phosphorylation of Ser262, Ser279/282, and Ser368. *FEBS Lett.* **588**, 836–844.
Frémont, S. and Echard, A. (2018). Membrane Traffic in the Late Steps of Cytokinesis. *Curr. Biol.* **28**, R458–R470.
Frolov, M. V and Dyson, N. J. (2004). Molecular mechanisms of E2F-dependent activation and pRB-mediated repression. *J. Cell Sci.* **117**, 2173–2181.
Fujii, S., Tanaka, H. and Hirano, T. (2017). Detection and characterization of individual endocytosis of AMPA-type glutamate receptor around postsynaptic membrane. *Genes to Cells* **22**, 583–590.
Fumagalli, M., Rossiello, F., Clerici, M., Barozzi, S., Cittaro, D., Kaplunov, J. M., Bucci, G., Dobreva, M., Matti, V., Beausejour, C. M., et al. (2012). Telomeric DNA damage is irreparable and causes persistent DNA-damage-response activation. *Nat. Cell Biol.* **14**, 355–365.
Gan, B. and DePinho, R. A. (2009). mTORC1 signaling governs hematopoietic stem cell quiescence. *Cell Cycle* **8**, 1003–1006.
Gan, Q. and Watanabe, S. (2018). Synaptic Vesicle Endocytosis in Different Model Systems. *Front. Cell. Neurosci.* **12**, 171.
García-Prat, L., Martínez-Vicente, M., Perdiguero, E., Ortet, L., Rodríguez-Ubreva, J., Rebollo, E., Ruiz-Bonilla, V., Gutarra, S., Ballestar, E., Serrano, A. L., et al. (2016). Autophagy maintains stemness by preventing senescence. *Nature* **529**, 37–42.
Gehne, N., Lamik, A., Lehmann, M., Haseloff, R. F., Andjelkovic, A. V. and Blasig, I. E. (2017). Cross-over endocytosis of claudins is mediated by interactions via their extracellular loops. *PLoS One* **12**, e0182106.
Georgiou, M., Marinari, E., Burden, J. and Baum, B. (2008). Cdc42, Par6, and aPKC Regulate Arp2/3-Mediated Endocytosis to Control Local Adherens Junction Stability. *Curr. Biol.* **18**, 1631–1638.
Ghossoub, R., Lindbæk, L., Molla-Herman, A., Schmitt, A., Christensen, S. T. and Benmerah, A. (2016). Morphological and Functional Characterization of the Ciliary Pocket by Electron and Fluorescence Microscopy. In *Cilia. Methods in Molecular Biology* (ed. Satir P., C. S.), pp. 35–51. Humana Press, New York, NY.
Goto, H., Inaba, H. and Inagaki, M. (2017). Mechanisms of ciliogenesis suppression in dividing cells. *Cell. Mol. Life Sci.* **74**, 881–890.
Gottesfeld, J. M. and Forbes, D. J. (1997). Mitotic repression of the transcriptional machinery. *Trends Biochem. Sci.* **22**, 197–202.
Gruenberg, J. and Van Der Goot, F. G. (2006). Mechanisms of pathogen entry through the endosomal compartments. *Nat. Rev. Mol. Cell Biol.* **7**, 495–504.
Guo, J., Longshore, S., Nair, R. and Warner, B. W. (2009). Retinoblastoma protein (pRb), but not p107 or p130, is required for maintenance of enterocyte quiescence and differentiation in small intestine. *J. Biol. Chem.* **284**, 134–40.
Hahn, A. T., Jones, J. T. and Meyer, T. (2009). Quantitative analysis of cell cycle phase durations and PC12 differentiation using fluorescent biosensors. *Cell Cycle* **8**, 1044–1052.
Hanahan, D. and Weinberg, R. A. (2011). Hallmarks of Cancer: The Next Generation. *Cell* **144**, 646–674.
Harashima, H., Dissmeyer, N. and Schnittger, A. (2013). Cell cycle control across the eukaryotic kingdom. *Trends Cell Biol.* **23**, 345–356.

Heck, B. W. and Devenport, D. (2017). Trans-endocytosis of Planar Cell Polarity Complexes during Cell Division. *Curr. Biol.* **27**, 3725–3733.e4.
Herrup, K. and Busser, J. C. (1995). The induction of multiple cell cycle events precedes target-related neuronal death. *Development* **121**, 2385–95.
Hinze, C. and Boucrot, E. (2018). Local actin polymerization during endocytic carrier formation. *Biochem. Soc. Trans.* **167**,.
Ho, T. T., Warr, M. R., Adelman, E. R., Lansinger, O. M., Flach, J., Verovskaya, E. V., Figueroa, M. E. and Passegué, E. (2017). Autophagy maintains the metabolism and function of young and old stem cells. *Nature* **543**, 205–210.
Holst, M. R., Vidal-Quadras, M., Larsson, E., Song, J., Hubert, M., Blomberg, J., Lundborg, M., Landström, M. and Lundmark, R. (2017). Clathrin-Independent Endocytosis Suppresses Cancer Cell Blebbing and Invasion. *Cell Rep.* **20**, 1893–1905.
Huang, H., Bae, C., Sachs, F. and Suchyna, T. M. (2013). Caveolae Regulation of Mechanosensitive Channel Function in Myotubes. *PLoS One* **8**, e72894.
Ikari, A., Takiguchi, A., Atomi, K. and Sugatani, J. (2011). Epidermal growth factor increases clathrin-dependent endocytosis and degradation of claudin-2 protein in MDCK II cells. *J. Cell. Physiol.* **226**, 2448–2456.
Ikonen, E. and Parton, R. G. (2000). Caveolins and cellular cholesterol balance. *Traffic* **1**, 212–217.
Inoue, K. and Ishibe, S. (2015). Podocyte endocytosis in the regulation of the glomerular filtration barrier. *Am. J. Physiol. - Ren. Physiol.* **309**, F398–F405.
Itahana, K., Dimri, G. P., Hara, E., Itahana, Y., Zou, Y., Desprez, P. Y. and Campisi, J. (2002). A role for p53 in maintaining and establishing the quiescence growth arrest in human cells. *J. Biol. Chem.* **277**, 18206–18214.
Johannes, L., Wunder, C. and Shafaq-Zadah, M. (2016). Glycolipids and Lectins in Endocytic Uptake Processes. *J. Mol. Biol.* **428**, 4792–4818.
Kaksonen, M. and Roux, A. (2018). Mechanisms of clathrin-mediated endocytosis. *Nat. Rev. Mol. Cell Biol.* **19**, 313–326.
Kao, A. W., Ceresa, B. P., Santeler, S. R. and Pessin, J. E. (1998). Expression of a dominant interfering dynamin mutant in 3T3L1 adipocytes inhibits GLUT4 endocytosis without affecting insulin signaling. *J. Biol. Chem.* **273**, 25450–25457.
Kaur, S., Fielding, A. B., Gassner, G., Carter, N. J. and Royle, S. J. (2014). An unmet actin requirement explains the mitotic inhibition of clathrin-mediated endocytosis. *Elife* **3**, e00829.
Koo, B. K., Spit, M., Jordens, I., Low, T. Y., Stange, D. E., Van De Wetering, M., Van Es, J. H., Mohammed, S., Heck, A. J. R., Maurice, M. M., et al. (2012). Tumour suppressor RNF43 is a stem-cell E3 ligase that induces endocytosis of Wnt receptors. *Nature* **488**, 665–669.
Kozera, L., White, E. and Calaghan, S. (2009). Caveolae act as membrane reserves which limit mechanosensitive I(Cl,swell) channel activation during swelling in the rat ventricular myocyte. *PLoS One* **4**, e8312.
Lasagni, L., Lazzeri, E., J. Shankland, S., Anders, H.-J. and Romagnani, P. (2013). Podocyte Mitosis - A Catastrophe. *Curr. Mol. Med.* **13**, 13–23.
Latella, L., Sacco, A., Pajalunga, D., Tiainen, M., Macera, D., D'Angelo, M., Felici, A., Sacchi, A. and Crescenzi, M. (2001). Reconstitution of Cyclin D1-Associated Kinase Activity Drives Terminally Differentiated Cells into the Cell Cycle. *Mol. Cell. Biol.* **21**, 5631–5643.
Lee, S. H., Liu, L., Wang, Y. T. and Sheng, M. (2002). Clathrin adaptor AP2 and NSF interact with overlapping sites of GluR2 and play distinct roles in AMPA receptor trafficking and hippocampal LTD. *Neuron* **36**, 661–674.
Legesse-Miller, A., Raitman, I., Haley, E. M., Liao, A., Sun, L. L., Wang, D. J., Krishnan, N., Lemons, J. M. S., Suh, E. J., Johnson, E. L., et al. (2012). Quiescent fibroblasts are protected from proteasome inhibition-mediated toxicity. *Mol. Biol. Cell* **23**, 3566–3581.
Leibfried, A., Fricke, R., Morgan, M. J., Bogdan, S. and Bellaiche, Y. (2008). Drosophila Cip4 and WASp Define a Branch of the Cdc42-Par6-aPKC Pathway Regulating E-Cadherin Endocytosis. *Curr. Biol.* **18**, 1639–1648.
Lemons, J. M. S., Feng, X.-J., Bennett, B. D., Legesse-Miller, A., Johnson, E. L., Raitman, I., Pollina, E. a, Rabitz, H. a, Rabinowitz, J. D. and Coller, H. a (2010). Quiescent fibroblasts exhibit high metabolic activity. *PLoS Biol.* **8**, e1000514.
Leontieva, O. V., Gudkov, A. V. and Blagosklonny, M. V (2010). Weak p53 permits senescence during cell cycle arrest. *Cell Cycle* **9**, 4323–4327.
Leontieva, O. V., Demidenko, Z. N., Gudkov, A. V. and Blagosklonny, M. V. (2011). Elimination of proliferating cells unmasks the shift from senescence to quiescence caused by rapamycin. *PLoS One* **6**, e26126.
Lin, Y.-H., Currinn, H., Pocha, S. M., Rothnie, A., Wassmer, T. and Knust, E. (2015). AP-2-complex-mediated endocytosis of Drosophila Crumbs regulates polarity by antagonizing Stardust. *J. Cell Sci.* **128**, 4538–4549.
Liu, H., Adler, A. S., Segal, E. and Chang, H. Y. (2007). A transcriptional program mediating entry into cellular quiescence. *PLoS Genet.* **3**, 0996-1008.
Liu, Y., Elf, S. E., Miyata, Y., Sashida, G., Liu, Y., Huang, G., Di Giandomenico, S., Lee, J. M., Deblasio, A., Menendez, S., et al. (2009). p53 Regulates Hematopoietic Stem Cell Quiescence. *Cell Stem Cell* **4**, 37–48.
Liu, L., Shi, H., Chen, X. and Wang, Z. (2011). Regulation of EGF-stimulated EGF receptor endocytosis during M phase. *Traffic* **12**, 201–17.
Liu, T.-L., Upadhyayula, S., Milkie, D. E., Singh, V., Wang, K., Swinburne, I. A., Mosaliganti, K. R., Collins, Z. M., Hiscock, T. W., Shea, J., et al. (2018). Observing the cell in its native state: Imaging subcellular dynamics in multicellular organisms. *Science (80-.).* **360**, eaaq1392.
Lo, H. P., Nixon, S. J., Hall, T. E., Cowling, B. S., Ferguson, C., Morgan, G. P., Schieber, N. L., Fernandez-Rojo, M. A., Bastiani, M., Floetenmeyer, M., et al. (2015). The caveolin-Cavin system plays a conserved and critical role in mechanoprotection of skeletal muscle. *J. Cell Biol.* **210**, 833–849.
Lundmark, R., Doherty, G. J., Howes, M. T., Cortese, K., Vallis, Y., Parton, R. G. and McMahon, H. T. (2008). The GTPase-Activating Protein GRAF1 Regulates the CLIC/GEEC Endocytic Pathway. *Curr. Biol.* **18**, 1802–1808.
Lyle, S. and Moore, N. (2011). Quiescent, slow-cycling stem cell populations in cancer: A review of the evidence and discussion of significance. *J. Oncol.* **2011**,.
Majoul, I., Schmidt, T., Pomasanova, M., Boutkevich, E., Kozlov, Y. and Söling, H.-D. (2002). Differential expression of receptors for Shiga and Cholera toxin is regulated by the cell cycle. *J. Cell Sci.* **115**, 817–26.
Maldonado-Báez, L., Williamson, C. and Donaldson, J. G. (2013). Clathrin-independent endocytosis: A cargo-centric view. *Exp. Cell Res.* **319**, 2759–2769.

Maritzen, T. and Haucke, V. (2017). Coupling of exocytosis and endocytosis at the presynaptic active zone. *Neurosci. Res.* **127**, 45–52.
Matsuda, T., Okamura, K., Sato, Y., Morimoto, A., Ono, M., Kohno, K. and Kuwano, M. (1992). Decreased response to epidermal growth factor during cellular senescence in cultured human microvascular endothelial cells. *J. Cell. Physiol.* **150**, 510–516.
McMahon, H. T. and Boucrot, E. (2011). Molecular mechanism and physiological functions of clathrin-mediated endocytosis. *Nat. Rev. Mol. Cell Biol.* **12**, 517–33.
Mercer, J. and Helenius, A. (2012). Gulping rather than sipping: Macropinocytosis as a way of virus entry. *Curr. Opin. Microbiol.* **15**, 490–499.
Miyashita, Y. and Ozawa, M. (2007). Increased internalization of p120-uncoupled E-cadherin and a requirement for a dileucine motif in the cytoplasmic domain for endocytosis of the protein. *J. Biol. Chem.* **282**, 11540–8.
Molla-Herman, A., Ghossoub, R., Blisnick, T., Meunier, A., Serres, C., Silbermann, F., Emmerson, C., Romeo, K., Bourdoncle, P., Schmitt, A., et al. (2010). The ciliary pocket: an endocytic membrane domain at the base of primary and motile cilia. *J. Cell Sci.* **123**, 1785–1795.
Morisco, C., Marrone, C., Galeotti, J., Shao, D., Vatner, D. E., Vatner, S. F. and Sadoshima, J. (2008). Endocytosis machinery is required for β1-adrenergic receptor-induced hypertrophy in neonatal rat cardiac myocytes. *Cardiovasc. Res.* **78**, 36–44.
Moulding, D. A., Blundell, M. P., Spiller, D. G., White, M. R. H., Cory, G. O., Calle, Y., Kempski, H., Sinclair, J., Ancliff, P. J., Kinnon, C., et al. (2007). Unregulated actin polymerization by WASp causes defects of mitosis and cytokinesis in X-linked neutropenia. *J. Exp. Med.* **204**, 2213–2224.
Muñoz-Espín, D. and Serrano, M. (2014). Cellular senescence: From physiology to pathology. *Nat. Rev. Mol. Cell Biol.* **15**, 482–496.
Muranen, T., Iwanicki, M. P., Curry, N. L., Hwang, J., DuBois, C. D., Coloff, J. L., Hitchcock, D. S., Clish, C. B., Brugge, J. S. and Kalaany, N. Y. (2017). Starved epithelial cells uptake extracellular matrix for survival. *Nat. Commun.* **8**, 13989.
Nagy, P., Kovács, L., Sándor, G. O. and Juhász, G. (2016). Stem-cell-specific endocytic degradation defects lead to intestinal dysplasia in Drosophila. *Dis. Model. Mech.* **9**, 501–512.
Nakayama, M., Nakayama, A., Van Lessen, M., Yamamoto, H., Hoffmann, S., Drexler, H. C. A., Itoh, N., Hirose, T., Breier, G., Vestweber, D., et al. (2013). Spatial regulation of VEGF receptor endocytosis in angiogenesis. *Nat. Cell Biol.* **15**, 249–260.
Nanes, B. A., Chiasson-MacKenzie, C., Lowery, A. M., Ishiyama, N., Faundez, V., Ikura, M., Vincent, P. A. and Kowalczyk, A. P. (2012). p120-catenin binding masks an endocytic signal conserved in classical cadherins. *J. Cell Biol.* **199**, 365–380.
Nimlamool, W., Andrews, R. M. K. and Falk, M. M. (2015). Connexin43 phosphorylation by PKC and MAPK signals VEGF-mediated gap junction internalization. *Mol. Biol. Cell* **26**, 2755–2768.
Nunez, M. T., Tapia, V. and Arredondo, M. (1996). Intestinal epithelia (Caco-2) cells acquire iron through the basolateral endocytosis of transferrin. *J. Nutr.* **126**, 2151–2158.
Olszewski, M. B., Chandris, P., Park, B. C., Eisenberg, E. and Greene, L. E. (2014). Disruption of Clathrin-Mediated Trafficking Causes Centrosome Overduplication and Senescence. *Traffic* **15**, 60–77.
Orthwein, A., Fradet-Turcotte, A., Noordermeer, S. M., Canny, M. D., Brun, C. M., Strecker, J., Escribano-Diaz, C. and Durocher, D. (2014). Mitosis inhibits DNA double-strand break repair to guard against telomere fusions. *Science (80-.).* **344**, 189–193.
Ottesen, A. H., Louch, W. E., Carlson, C. R., Landsverk, O. J. B., Kurola, J., Johansen, R. F., Moe, M. K., Aronsen, J. M., Høiseth, A. D., Jarstadmarken, H., et al. (2015). Secretoneurin is a novel prognostic cardiovascular biomarker associated with cardiomyocyte calcium handling. *J. Am. Coll. Cardiol.* **65**, 339–351.
Palacios, F., Schweitzer, J. K., Boshans, R. L. and D'Souza-Schorey, C. (2002). ARF6-GTP recruits Nm23-H1 to facilitate dynamin-mediated endocytosis during adherens junctions disassembly. *Nat. Cell Biol.* **4**, 929–936.
Park, W. Y., Park, J. S., Cho, K. A., Kim, D. I., Ko, Y. G., Seo, J. S. and Park, S. C. (2000). Up-regulation of caveolin attenuates epidermal growth factor signaling in senescent cells. *J. Biol. Chem.* **275**, 20847–20852.
Park, J. S., Park, W. Y., Cho, K. A., Kim, D. I., Jhun, B. H., Kim, S. R. and Park, S. C. (2001). Down-regulation of amphiphysin-1 is responsible for reduced receptor-mediated endocytosis in the senescent cells. *FASEB J.* **15**, 1625–1627.
Parton, R. G., Tillu, V. A. and Collins, B. M. (2018). Caveolae. *Curr. Biol.* **28**, R402–R405.
Pedersen, L. B., Mogensen, J. B. and Christensen, S. T. (2016). Endocytic Control of Cellular Signaling at the Primary Cilium. *Trends Biochem. Sci.* **41**, 784–797.
Pypaert, M., Lucocq, J. M. and Warren, G. (1987). Coated pits in interphase and mitotic A431 cells. *Eur J Cell Biol* **45**, 23–9.
Qin, X.-S., Tsukaguchi, H., Shono, A., Yamamoto, A., Kurihara, H. and Doi, T. (2009). Phosphorylation of Nephrin Triggers Its Internalization by Raft-Mediated Endocytosis. *J. Am. Soc. Nephrol.* **20**, 2534–2545.
Radeva, M. Y. and Waschke, J. (2018). Mind the gap: mechanisms regulating the endothelial barrier. *Acta Physiol.* **222**, e12860.
Rajarajacholan, U. K., Thalappilly, S. and Riabowol, K. (2013). The ING1a Tumor Suppressor Regulates Endocytosis to Induce Cellular Senescence Via the Rb-E2F Pathway. *PLoS Biol.* **11**, e1001502.
Richmond, C. A., Shah, M. S., Carlone, D. L. and Breault, D. T. (2016). Factors regulating quiescent stem cells: insights from the intestine and other self-renewing tissues. *J. Physiol.* **594**, 4805–4813.
Rodriguez-Boulan, E., Kreitzer, G. and Müsch, A. (2005). Organization of vesicular trafficking in epithelia. *Nat. Rev. Mol. Cell Biol.* **6**, 233–247.
Rosendale, M., Jullié, D., Choquet, D. and Perrais, D. (2017). Spatial and Temporal Regulation of Receptor Endocytosis in Neuronal Dendrites Revealed by Imaging of Single Vesicle Formation. *Cell Rep.* **18**, 1840–1847.
Royle, S. J., Bright, N. A. and Lagnado, L. (2005). Clathrin is required for the function of the mitotic spindle. *Nature* **434**, 1152–1157.
Sager, P. R., Brown, P. A. and Berlin, R. D. (1984). Analysis of transferrin recycling in mitotic and interphase hela cells by quantitative fluorescence microscopy. *Cell* **39**, 275–282.
Santos, A. J. M., Meinecke, M., Fessler, M. B., Holden, D. W. and Boucrot, E. (2013). Preferential invasion of mitotic cells by Salmonella reveals that cell surface cholesterol is maximal during metaphase. *J. Cell Sci.* **126**, 2990–2996.
Schmid, S. L. (2017). Reciprocal regulation of signaling and endocytosis: Implications for the evolving cancer cell. *J. Cell Biol.* **216**, 2623–2632.

Schöneberg, J., Dambournet, D., Liu, T.-L., Forster, R., Hockemeyer, D., Betzig, E. and Drubin, D. G. (2018). 4D cell biology: big data image analytics and lattice light-sheet imaging reveal dynamics of clathrin-mediated endocytosis in stem cell derived intestinal organoids. *Mol. Biol. Cell* mbc.E18-06-0375.

Segrelles, C., García-Escudero, R., Garín, M. I., Aranda, J. F., Hernández, P., Ariza, J. M., Santos, M., Paramio, J. M. and Lorz, C. (2014). Akt signaling leads to stem cell activation and promotes tumor development in epidermis. *Stem Cells* **32**, 1917–1928.

Seshadri, T. and Campisi, J. (1990). Repression of c-fos transcription and an altered genetic program in senescent human fibroblasts. *Science (80-.).* **247**, 205–209.

Shapiro, H. M. (1981). Flow cytometric estimation of DNA and RNA content in intact cells stained with Hoechst 33342 and pyronin Y. *Cytometry* **2**, 143–50.

Sharpless, N. E. and Sherr, C. J. (2015). Forging a signature of in vivo senescence. *Nat. Rev. Cancer* **15**, 397–408.

Shigematsu, S., Watson, R. T., Khan, A. H. and Pessin, J. E. (2003). The adipocyte plasma membrane caveolin functional/structural organization is necessary for the efficient endocytosis of GLUT4. *J. Biol. Chem.* **278**, 10683–10690.

Shrestha, R., Little, K. A., Tamayo, J. V, Li, W., Perlman, D. H. and Devenport, D. (2015). Mitotic Control of Planar Cell Polarity by Polo-like Kinase 1. *Dev. Cell* **33**, 522–34.

Snijder, B., Sacher, R., Rämö, P., Damm, E. M., Liberali, P. and Pelkmans, L. (2009). Population context determines cell-to-cell variability in endocytosis and virus infection. *Nature* **461**, 520–523.

Snyder, J. C., Rochelle, L. K., Ray, C., Pack, T. F., Bock, C. B., Lubkov, V., Lyerly, H. K., Waggoner, A. S., Barak, L. S. and Caron, M. G. (2017). Inhibiting clathrin-mediated endocytosis of the leucine-rich G protein-coupled receptor-5 diminishes cell fitness. *J. Biol. Chem.* **292**, 7208–7222.

Soda, K., Balkin, D. M., Ferguson, S. M., Paradise, S., Milosevic, I., Giovedi, S., Volpicelli-Daley, L., Tian, X., Wu, Y., Ma, H., et al. (2012). Role of dynamin, synaptojanin, and endophilin in podocyte foot processes. *J. Clin. Invest.* **122**, 4401–4411.

Soeiro, M. de N. C., Mota, R. A., Batista, D. da G. J. and Meirelles, M. de N. L. (2002). Endocytic pathway in mouse cardiac cells. *Cell Struct. Funct.* **27**, 469–478.

Sousa-Victor, P., Gutarra, S., García-Prat, L., Rodriguez-Ubreva, J., Ortet, L., Ruiz-Bonilla, V., Jardí, M., Ballestar, E., González, S., Serrano, A. L., et al. (2014). Geriatric muscle stem cells switch reversible quiescence into senescence. *Nature* **506**, 316–321.

Stamatovic, S. M., Johnson, A. M., Sladojevic, N., Keep, R. F. and Andjelkovic, A. V. (2017). Endocytosis of tight junction proteins and the regulation of degradation and recycling. *Ann. N. Y. Acad. Sci.* **1397**, 54–65.

Swildens, J., De Vries, A. A. F., Li, Z., Umar, S., Atsma, D. E., Schalij, M. J. and Van Der Laarse, A. (2010). Integrin stimulation favors uptake of macromolecules by cardiomyocytes in vitro. *Cell. Physiol. Biochem.* **26**, 999–1010.

Tacheva-Grigorova, S. K., Santos, A. J. M., Boucrot, E. and Kirchhausen, T. (2013). Clathrin-mediated endocytosis persists during unperturbed mitosis. *Cell Rep.* **4**, 659–68.

Taylor, M. J., Perrais, D. and Merrifield, C. J. (2011). A high precision survey of the molecular dynamics of mammalian clathrin-mediated endocytosis. *PLoS Biol.* **9**, e1000604.

Terzi, M. Y., Izmirli, M. and Gogebakan, B. (2016). *The cell fate: senescence or quiescence*. Springer Netherlands.

Thorn, H., Stenkula, K. G., Karlsson, M., Örtegren, U., Nystrom, F. H., Gustavsson, J. and Strålfors, P. (2003). Cell Surface Orifices of Caveolae and Localization of Caveolin to the Necks of Caveolae in Adipocytes. *Mol. Biol. Cell* **14**, 3967–3976.

Tojima, T. and Kamiguchi, H. (2015). Exocytic and endocytic membrane trafficking in axon development. *Dev. Growth Differ.* **57**, 291–304.

Veiga, E. and Cossart, P. (2005). Listeria hijacks the clathrin-dependent endocytic machinery to invade mammalian cells. *Nat. Cell Biol.* **7**, 894–900.

Veiga, E., Guttman, J. A., Bonazzi, M., Boucrot, E., Toledo-Arana, A., Lin, A. E., Enninga, J., Pizarro-Cerdá, J., Finlay, B. B., Kirchhausen, T., et al. (2007). Invasive and Adherent Bacterial Pathogens Co-Opt Host Clathrin for Infection. *Cell Host Microbe* **2**, 340–351.

von Muhlinen, N., Horikawa, I., Alam, F., Isogaya, K., Lissa, D., Vojtesek, B., Lane, D. P. and Harris, C. C. (2018). p53 isoforms regulate premature aging in human cells. *Oncogene* **37**, 1–15.

Warren, G., Davoust, J. and Cockcroft, A. (1984). Recycling of transferrin receptors in A431 cells is inhibited during mitosis. *Embo J* **3**, 2217–25.

Watanabe, S. and Boucrot, E. (2017). Fast and ultrafast endocytosis. *Curr. Opin. Cell Biol.* **47**, 64–71.

Watanabe, S., Mamer, L., Raychaudhuri, S., Luvsanjav, D., Eisen, J., Trimbuch, T., Sohl-Kielczynski, B., Fenske, P., Milosevic, I., Rosenmund, C., et al. (2018). Synaptojanin and Endophilin Mediate Neck Formation during Ultrafast Endocytosis. *Neuron* **98**, 1184–1197.e6.

Waxse, B. J., Sengupta, P., Hesketh, G. G., Lippincott-Schwartz, J. and Buss, F. (2017). Myosin VI facilitates connexin 43 gap junction accretion. *J. Cell Sci.* **130**, 827–840.

Wei, J. H. and Seemann, J. (2009). Mitotic division of the mammalian Golgi apparatus. *Semin. Cell Dev. Biol.* **20**, 810–816.

Wei, H., Geng, J., Shi, B., Liu, Z., Wang, Y.-H., Stevens, A. C., Sprout, S. L., Yao, M., Wang, H. and Hu, H. (2016). Cutting Edge: Foxp1 Controls Naive CD8 + T Cell Quiescence by Simultaneously Repressing Key Pathways in Cellular Metabolism and Cell Cycle Progression. *J. Immunol.* **196**, 3537–3541.

Wheaton, K., Sampsel, K., Boisvert, F. M., Davy, A., Robbins, S. and Riabowol, K. (2001). Loss of functional caveolae during senescence of human fibroblasts. *J. Cell. Physiol.* **187**, 226–35.

Yamaki, T., Kamiya, Y., Ohtake, K., Uchida, M., Seki, T., Ueda, H., Kobayashi, J., Morimoto, Y. and Natsume, H. (2014). A mechanism enhancing macromolecule transport through paracellular spaces induced by poly-L-arginine: Poly-L-arginine induces the internalization of tight junction proteins via clathrin-mediated endocytosis. *Pharm. Res.* **31**, 2287–2296.

Yerbury, J. J. (2016). Protein aggregates stimulate macropinocytosis facilitating their propagation. *Prion* **10**, 119–126.

Yue, F., Bi, P., Wang, C., Shan, T., Nie, Y., Ratliff, T. L., Gavin, T. P. and Kuang, S. (2017). Pten is necessary for the quiescence and maintenance of adult muscle stem cells. *Nat. Commun.* **8**, 14328.

Yusuf, I. and Fruman, D. A. (2003). Regulation of quiescence in lymphocytes. *Trends Immunol.* **24**, 380–386.

Zanoni, P., Velagapudi, S., Yalcinkaya, M., Rohrer, L. and von Eckardstein, A. (2018). Endocytosis of lipoproteins. *Atherosclerosis* **275**, 273–295.

Box 1. Brief overview of the main endocytic pathways.

Endocytic pathways are differentiated by the shape, size and kinetics of the carriers produced, the cargoes internalized and the cytosolic proteins marking and regulating them. Clathrin-mediated endocytosis (CME) is constitutively active and is the best characterized process (Kaksonen and Roux, 2018). Many receptors, including transferrin receptor (TfR), mostly rely on CME to enter cells. Clathrin chains assemble into triskelia that, once recruited by AP2 or other adaptors, polymerize into a proteinaceous coat around the nascent vesicles. The GTPase Dynamin then severs the neck of budding clathrin-coated pits (Kaksonen and Roux, 2018). Several Clathrin-independent endocytic (CIE) processes exist in parallel to CME and mediate the uptake of cargoes that do not use CME selectively. These include CD44, CD147, MHC class I, interleukin-2 receptor (IL2R) and β1-adrenergic receptor or glycosylphosphatidylinositol (GPI)-anchored proteins, such as CD55, CD59 or CD90 (also called Thy-1) (Maldonado-Báez et al., 2013). They also regulate specific processes, such as the fast removal of cell-surface receptors, response to receptor hyper-stimulation or stress hormones ('fight or flight' response) (Johannes et al., 2015; Ferreira and Boucrot, 2018). Some of the CIE processes include the Clathrin-independent carrier/GPI-anchored proteins-enriched carriers (CLIC/GEEC) pathway, which generates endocytic carriers using the BAR domain proteins GRAF1, Irsp53 and PICK1, as well as local actin polymerization that is mediated by Arf1, its GEF GBF1 and Cdc42, but not Dynamin (reviewed in Lundmark et al., 2008, Hinze and Boucrot, 2018). Initial membrane curvature in some CIE events is mediated by a mechanism termed the 'glycolipid-lectin (GL-Lect) hypothesis', whereby the clustering of extracellular cargo proteins or lipids by galectin-3 or Shiga and cholera toxins drives an inward-directed buckling of the membrane (reviewed in Johannes et al., 2016). Fast endophilin-mediated endocytosis (FEME) is not constitutive, but promptly forms tubulo-vesicular endocytic carriers following the activation of several receptors by their cognate ligands (reviewed in Watanabe and Boucrot, 2017; Ferreira and Boucrot, 2018). Ultrafast endocytosis at the synapse shares features with FEME in that it also relies on Dynamin, Endophilin, actin and Synaptojanin, but is at least one order of magnitude faster (reviewed in Gan and Watanabe, 2018; Watanabe and Boucrot, 2017). Following intense stimulations, macropinocytosis and activity-dependent bulk endocytosis (ADBE) in neurons form large (≥ 0.5μm) carriers that take up substantial amounts of extracellular material and plasma membrane (Cousin, 2017; Mercer & Helenius, 2012). Finally, caveolae are cholesterol-rich membrane domains on the plasma membrane that invaginate and pinch off upon clustering of Caveolin and Cavin proteins (Parton et al., 2018).

Box 2. Cell cycle exit.

Proliferating cells progress through interphase (G1, S and G2) and divide during mitosis (Harashima et al., 2013), which is divided in five successive steps: prophase, metaphase, anaphase, telophase and cytokinesis. Because of different lengths of time spent by mammalian cells at each stage, an asynchronous population has typically >98% of cells in interphase (~40-50% of cells in G1, ~20-30% in S and ~10-20% in G2) and 0.5-2% of cells undergoing mitosis (Cameron and Greulich, 1963; Hahn et al., 2009). Continuous proliferation is not physiologically sustainable, and most cells in an adult multicellular organism exit the cell cycle temporally (quiescence) or irreversibly (terminally differentiation and senescence).

Cellular quiescence (also called G0) is the reversible exit from the cell cycle that is induced upon contact inhibition, mitogen withdrawal or cell isolation in suspension (Coller et al., 2006). Quiescent cells are resistant to differentiation and show increased survival (Coller et al., 2006; Cheung and Rando, 2013). Cell cycle exit is regulated by retinoblastoma proteins (Rb), which repress E2F-mediated transcription of cell cycle-progressing genes (Frolov and Dyson, 2004). Quiescent cells display reduced Akt (Segrelles et al., 2014) and increased PTEN phosphatase activity (Yue et al., 2017), which, in turn, suppress mTOR signaling (Gan and DePinho, 2009). Low mTOR activity protects quiescent cells from senescence and mediates the recycling of proteins and damaged organelles by autophagy, which is essential for long-term survival (García-Prat et al., 2016). Cell cycle-inhibiting genes, including p21, p27 and p53, are elevated in G0 cells (Coller et al., 2006; Itahana et al., 2002; H. Liu, Adler, Segal and Chang, 2007), whereas senescence-inducing p16 is suppressed (Leontieva et al., 2010; Sousa-Victor et al., 2014).

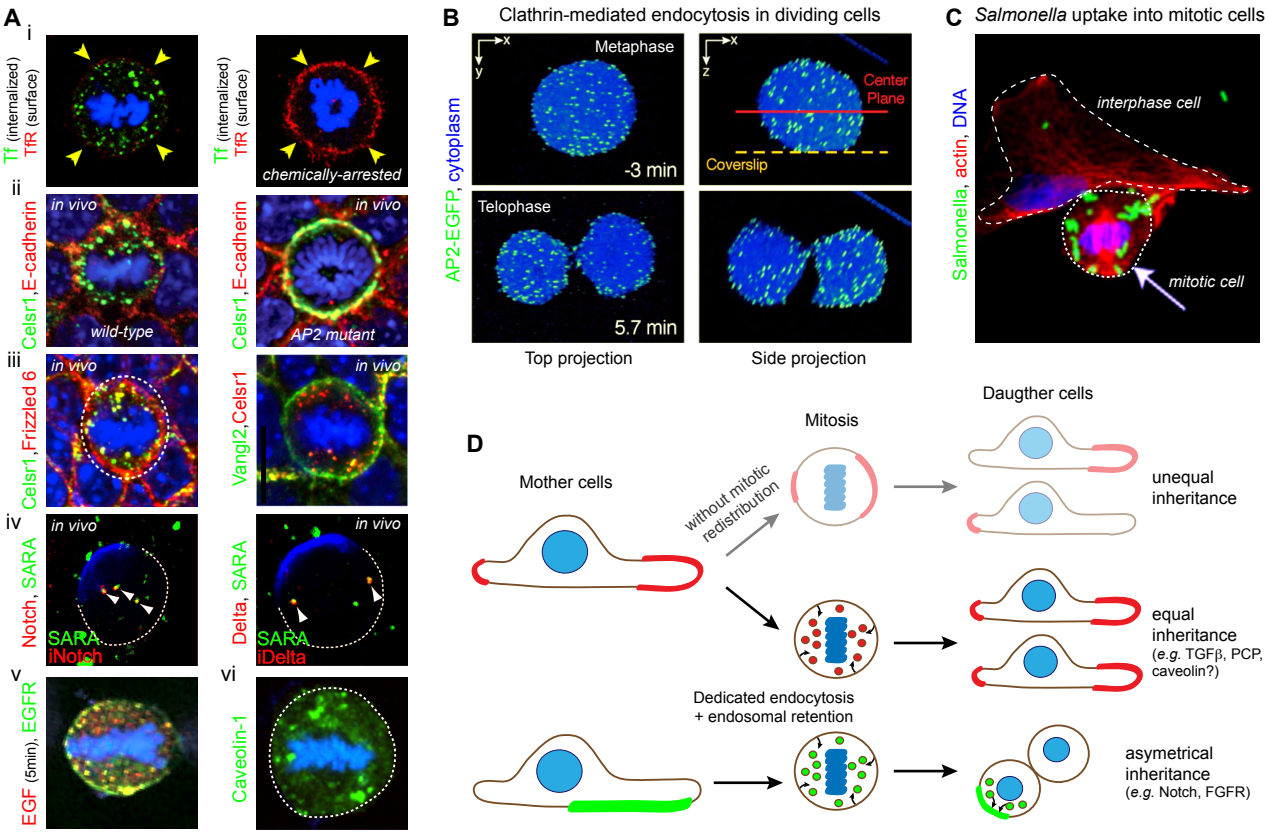
Cellular senescence is a growth arrest mechanism to prevent the replication of old or damaged cells (Muñoz-Espín and Serrano, 2014). Irreversible DNA damage, severe oxidative stress or telomere attrition induce senescence (Fumagalli et al., 2012), which is characterized by apoptosis resistance and hypo-responsiveness towards growth factors and other external stimuli (Matsuda et al., 1992; Seshadri and Campisi, 1990). Dependent on the trigger, senescence is either induced by the upregulation of the p53-$p21^{CIP1}$ axis or by the activation of the $p16^{INK4a}$-Rb pathway (Campisi, 2005; von Muhlinen et al., 2018). In contrast to quiescent cells, which are also characterized by high p53 activity, senescent cells retain a high mTOR activity and cellular growth (Leontieva et al., 2010; Leontieva et al., 2011).

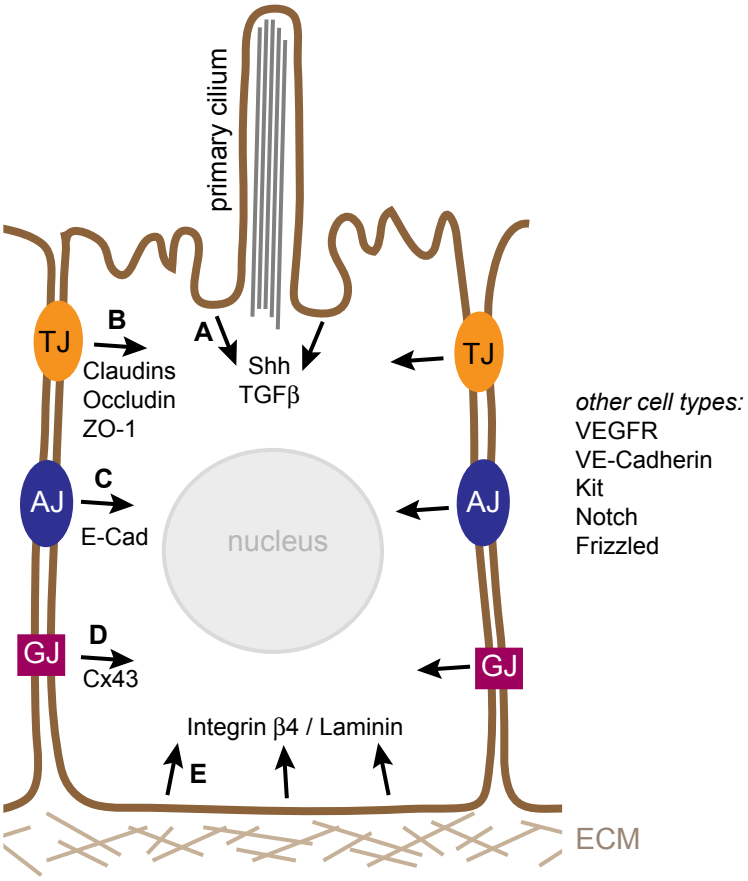
Finally, terminally differentiated cells, also called post-mitotic, are derived from pluripotent progenitors and are highly specialized cells that have permanently lost the capacity to replicate. There is no universal marker known for these cells, they are instead identified by markers that are specific to their differentiation lineage (Buttitta and Edgar, 2007).

Figure legends

Fig. 1. Endocytosis in dividing cells. A. Examples of endocytosis of endogenous cargoes during mitosis. (**i**) *Left*, Transferrin uptake (Tf, green) occurred in unperturbed mitotic BSC-1 cells, albeit at a much lower level than interphase. This is because Tf receptor available at the surface (TfR, red) is strongly reduced because the receptor is trapped in endosomes (not labeled) of mitotic cells. *Right*, Transferrin uptake (green) is blocked in nocodazole-arrested metaphase BSC-1 cells (chemical synchronization a common method to enrich mitotic cells), despite ample cell surface TfR (red). This is because chemical synchronization induces the disappearance of Clathrin-coated pits. Modified with permission from (Tacheva-Grigorova et al., 2013). (**ii, iii**) Planar Cell Polarity (PCP) receptors Celsr1 (ii), Frizzled (iii, left) and Vangl2 (iii, right) are actively endocytosed in mouse mitotic cells *in vivo.* A Celsr1 receptor bearing a mutation in its cytoplasmic tail (abrogating its interaction with AP2, '*AP2 mutant'*) is not internalized (ii, right). Modified from (Devenport et al., 2011 and Heck and Devenport, 2017). (i**v**) Notch (red, *left*) and its ligand Delta (red, *right*) are both internalized in dividing fly stem cells *in vivo* and accumulate into endosomes (SARA, green). Modified from (Coumailleau et al., 2009). (**v**) Both EGF (red) and EGFR (green) are internalized in a Clathrin-independent manner in mitotic COS-7 cells. Modified from (Liu et al., 2011). (**vi**) Most of the Caveolin-1 is internalized in mitotic BSC-1 cells and accumulates into endosomes. Modified from (Boucrot et al., 2011). (**B**) **Clathrin-mediated endocytosis in dividing cells.** Active Clathrin-coated pits (labeled with gene-edited AP2-EGFP, green) can be seen over the surface of the same cell during metaphase (*top*) and telophase (*bottom*). The cell cytoplasm is shown in blue. Modified from (Aguet et al., 2016). (**C**) **Efficient uptake of Salmonella into mitotic cells.** Salmonella (green) internalizes more efficiently into mitotic cells (arrow) than into interphase cells. Modified from (Santos et al., 2013). (**D**) **Model for the roles of endocytosis during cell division in the inheritance of transmembrane cell surface proteins.** Without mitotic redistribution, receptors that are polarized in the mother cells (e.g. PCP complex, TGFβ, Caveolin-1) would be inherited unequally between the two daughter cells, causing loss of polarity. Dedicated endocytosis during mitosis coupled with a shut-down of endosomal recycling causes receptors to accumulate in endosomes). The symmetrical partitioning of the endosomes between the two daughter cells mediates the equal inheritance of the proteins. Targeting of mitotic endosomes containing receptors (*e.g.* Notch, FGFR) into one of the daughter cell during asymmetrical division drives the maintenance of the stemness and the differentiation of the other cell during organ development.

Fig. 2. Endocytosis in quiescent cells. Illustrated here are examples of cargoes that are internalized in quiescent epithelial cells. (**A**) At the ciliary pocket, Sonic Hedgehog (Shh) and TGFβ are both internalized by Clathrin-mediated endocytosis (CME). (**B**) At tight junctions (TJs), endocytosis of Claudins, Occludin and ZO-1 is key to TJs maintenance. (**C**) E-cadherins mediate the interactions between cells through the formation adherens junctions (AJs); they are internalized by CME when in their free form and perhaps through a modified mechanism when clustered into AJs. (**D**) At gap junctions (GJs), Connexin 43 enters cells upon GJ disassembly. (**E**) Integrin β4 and its ligand Laminin from the extracellular matrix (ECM) are endocytosed and degraded into quiescent cells to provide amino acids and support their metabolism. Other receptors, such as VEGFR, VE-Cadherin, Kit, Notch and Frizzled are internalized into other quiescent cell types, such as endothelial or stem cells.


A
i
Tf (internalized)
TfR (surface)
chemically-arrested
ii
Celsr1,E-cadherin
in vivo
wild-type
AP2 mutant
iii
Celsr1,Frizzled 6
Vangl2,Celsr1
iv
Notch, SARA
SARA
iNotch
Delta, SARA
iDelta
v
EGF (5min), EGFR
vi
Caveolin-1
B
Clathrin-mediated endocytosis in dividing cells
AP2-EGFP, cytoplasm
Metaphase
-3 min
Center Plane
Coverslip
Telophase
5.7 min
Top projection
Side projection
C
Salmonella uptake into mitotic cells
Salmonella, actin, DNA
interphase cell
mitotic cell
D
Daugther cells
Mitosis
Mother cells
without mitotic redistribution
unequal inheritance
equal inheritance
(e.g. TGFβ, PCP, caveolin?)
Dedicated endocytosis
+ endosomal retention
asymetrical inheritance
(e.g. Notch, FGFR)


primary cilium
TJ
B
Claudins
Occludin
ZO-1
A
Shh
TGFβ
TJ
AJ
C
E-Cad
nucleus
AJ
GJ
D
Cx43
GJ
Integrin β4 / Laminin
E
ECM
other cell types:
VEGFR
VE-Cadherin
Kit
Notch
Frizzled