Endocytic Pathways

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Endocytosis is a shared process by which molecules, proteins, lipids, and liquids are sorted inside the cell via formation of intermediate vesicles. Vesicle formation occurs at the plasma membrane, where ligand receptors, binding proteins, and structural proteins are localized. After their internalization, the vesicles containing protein receptors or soluble molecules undergo a round of recycling, eventually leading to the fusion of the vesicle with an intracellular organelle. Such a process is an essential hallmark in all cell types—it regulates major cellular functions such as antigen presentation, intracellular signaling cascades, cell polarity, and synaptic transmission. Moreover, it is required to remove aged and dead cells from the body and is part of the defense against microbes. Given its importance, it is not surprising that even subtle perturbations affecting the endocytic machinery often impair cell function and cause several pathological conditions, such as cancer, and neurological and storage diseases. Finally, endocytosis represents an important cellular route for targeted drug-delivery in many diseases.

Keywords: endocytosis ; protein receptor ; disease ; fluorescence ; electron microscopy

1. Dynamin-Dependent Pathways

1.1. Clathrin-Dependent Endocytosis

Clathrin-mediated endocytosis (CME) represents the most characterized internalization pathway [1]. It occurs in all mammalian cells and is the principal route for cells to obtain nutrients; for example, facilitating the uptake of iron (via transferrin) and cholesterol (via low-density lipoproteins) [2].

Although historically considered a receptor-induced process, it is now known that clathrin coats can spontaneously assemble at the PM and that cargo-to-clathrin interactions are important for the stabilization of the process. Clathrin-coated pits occupy 0.5–2% of the cell surface and provide the membrane-deforming scaffold, which is fundamental in shaping the coated pit at the plasma membrane [3]. At the PM, clathrin assembles into a trimer of heterodimers, each unit consisting of one heavy and one light chain forming a triskelion [4]. Triskelia assembly lead to the formation of a lattice-like structure around the vesicles and such assembly is coordinated by several cargo-binding adaptor complexes; the most known of these is represented by the adaptor protein complex 2 (AP2), which is part of a wider family of hetero-tetrameric adaptor complexes (AP1-5). AP2 binds both to clathrin and protein cargoes via a peptide motif in their cytoplasmic domains. Alternatively, clathrin adaptors can recruit client cargoes more selectively [5]. These events are also strongly regulated by local actin and phosphoinositides on the plasma membrane. For instance, protein receptors clustering and phosphorylation recruits adaptin proteins at the plasma membrane, which initiates a cascade of low-affinity protein–protein and protein–lipid interactions (particularly with phosphatidylinositol 4,5-bisphosphate, PtdIns(4,5)P2), leading to the formation of a clathrin-coated pit (Smith et al., 2017). This is a highly dynamic and cooperative system in which a multitude of interactions form a pit within 30–120 s of ligand binding [6]. Clathrin-coated pits at the cell surface are highly diverse and with respect to the usage of adaptor and associated proteins [7]. This creates distinct microenvironments for the regulated entry of specific combinations of cargoes [3][8]. Moreover, ligand concentration also affects the mode of this endocytic route. For example, EGF is generally internalized by a clathrin-dependent endocytic route, but at higher concentrations, it enter cells through clathrin-independent routes [8]. Once formed, the pit rapidly invaginates to form a clathrin-coated vesicle, which pinches off the plasma membrane through the activity of dynamin, a large mechanical GTPase. PtdIns(4,5)P2 phosphatases, notably synaptojanin, complete the vesicle cycle by uncoating the vesicles [9]. Several viral pathogens such as the recent SARS-CoV-2 coronavirus and well-characterized families of virus (e.g., alpha-, rhabdo-, flavi-, picorna-, pox-, and adenoviruses, among others) enter cells by clathrin-mediated endocytosis, targeting receptors, or machineries internalized by the clathrin-dependent pathway [10][11]. For some pathogens, this route is obligatory, and for others, CME is one of the available escaping routes. Bacteria and large particles up to 1 µm in diameter have also been shown to co-opt clathrin and form actin-rich pedestals to facilitate their uptake [11]. Although longer than the diameter of the typical clathrin-coated vesicle, these pathogens can be internalized by CME through the actin

elongation of the clathrin-coated pit [12]. The requirement for actin recruitment, although, can slow the endocytic process, leading to altered internalization kinetics, compared to conventional CME [13].

1.2. Fast Endophilin-Mediated Endocytosis (FEME)

FEME recently emerged as a novel fast endocytic pathway of specific membrane receptors, which are important in cell migration and growth factor signaling [14]. Cargoes that follow FEME include β1-adrenergic, dopaminergic, and acetylcholine receptors; the IL-2 receptor and growth factor receptors (EGFR, HGFR); and toxins such as CTxB and STxB. However, so far, only the β1-adrenergic receptor relies on FEME. The FEME pathway is clathrin-independent, but dynamin dependent. Unlike CME, FEME is not constitutive but is rapidly triggered by binding of receptors by their ligands. Similar to CME, FEME requires a pre-enrichment of its main component, endophilin-A2, into discrete clusters on the plasma membrane prior to receptor activation. Such protein interactions recruit the PtdIns(3,4)P2-binding protein lamellipodin, which stabilize endophilin at the edge of the migrating cells. Formation of FEME carriers is extremely rapid (<10 s). Upon receptor activation, indeed, the direct interaction between the SH3 domain of endophilin and cargoes or the indirect association through intermediate proteins such as CIN85 and Cbl activate a cascade of intracellular signaling, culminating in the formation of a 60–80 nm tubular invagination [13]. In the absence of receptor activation, endophilin spots are rapidly unclustered from the plasma membrane. Recently, Cdk5 and GSK3β were identified as key negative regulators of FEME, allowing the cells rapid uptake by the pathway only when their activity is low. Indeed, Cdk5 and GSK3β antagonize the binding of Endophilin to Dynamin-1 and to CRMP4 for local regulation of FEME [15].

1.3. EGFR Non-Clathrin Endocytosis

Although traditionally internalized by CME, EGFR receptors can be internalized by alternative endocytic routes in an activity-dependent manner. High concentrations of EGF (>2 ng/mL) can trigger the EGFR receptor to enter cancer cells using an unconventional clathrin-independent pathway called EGFR-NCE identified by light and electron microscopy [16] [17]. At even higher EGF concentrations (>50 ng/mL), both FEME and micropinocytosis mediates rapid EGFR internalization from the cell surface required to protect cells from excessive ERK and AKT signaling [18]. EGFR-NCE occurs via the mono-ubiquitination of EGFR and the release of IP3-mediated Ca^{2+} release stored in the endoplasmic reticulum (ER), which triggers the carrier formation. Moreover, the co-internalization of at least one CD147 receptor is required to internalize EGFR via EGFR-NCE [18][19].

1.4. Ultrafast Endocytosis (UFE)

Ultrafast endocytosis (UFE) is a rapid endocytic route for synaptic vesicles, which have been recently observed both in primary neurons and acute brain slices [20]. UFE occurs within 100 ms from the end of an action potential and generates several 80 nm small and elongated cisternae at the plasma membrane, in proximity of the fusion site [21]. Once internalized, cisternae fuse with synaptic endosomes from which synaptic vesicle are reformed. This process is clathrin-mediated [22]. Although most of the mechanisms that govern UFE are still not fully understood, it is known that (1) UFE is triggered by Ca^{2+}, (2) is sensitive to membrane tension and that (3) endophilin, synaptojanin, and dynamin, as well as actin, are important for cisternae membrane curvature [9]. Moreover, local protein organization, as well as lipid composition favoring membrane fluidity, are likely to support UFE [23]. Putative UFE cargoes are represented by synaptic vesicle proteins, which are critical for vesicle function and need thus to be recycled rapidly, such as SNAREs or glutamate transporters. However, how such cargoes are sorted back to the cisternae is still unknown.

1.5. Activity-Dependent Bulk Endocytosis (ADBE)

In neurons, CME and ultrafast endocytosis (UFE) recycle synaptic vesicles in response to low to moderate frequency of action potentials. During sustained neuronal activity, a different endocytic process called activity dependent bulk endocytosis (ADBE) takes over. ADBE resembles micropinocytosis but is triggered by elevated local calcium within synaptic terminals and a high amount of exocytosed membrane [24]. This process is dynamically regulated by two kinases, Cdk5 and Glycogen Synthase Kinase 3b (GSK3b), which inhibit ADBE modulators in resting neurons. During high neuronal activity, the local increase in Ca^{2+} activates the phosphatase calcineurin, which in turn activates ADBE modulators [25]. Once triggered, ADBE generates large actin-driven membrane invaginations, namely bulk endosomes, at the plasma membrane, where newly-released synaptic vesicle cargoes are located. VAMP4 is the main ADBE cargo, but many other synaptic vesicle proteins can be nonspecifically retrieved on the large (up to 500 nm) bulk endosomes [26].

2. Dynamin-Independent Pathways

A growing number of endocytic pathways do not rely on coat proteins or on a pinching system. Most of these pathways do not need coat assembly even during the endocytic intermediate steps. This is guaranteed by the involvement of lipid or protein components, which are sufficient to initiate membrane deformation without defined coat proteins. Shiga toxin entry, for instance, is one such example where binding of toxin to the ganglioside, Gb3, induces invaginations in cells as well as model membranes [27]. Similarly, endocytosis of many lipid-anchored proteins such as glycosylphosphatidylinositol-anchored proteins (GPI-APs) also does not appear to require any of the well-characterized coat proteins [28]. However, the GPI-anchored prion protein (PrP) might be internalized through both CIE and CME endocytosis, depending on the expression of the LRP-1 receptor, which drives PrP to CME [29][30].

2.1. Clathrin-Independent/Dynamin-Independent Endocytosis, CLICs/GEEC

Clathrin-independent carrier (CLIC) endocytosis/glycosylphosphatidylinositol-anchored protein enriched early endocytic compartment (GEEC) endocytosis is a cholesterol/GRAF1-dependent, but clathrin and dynamin-independent endocytic route [31][32]. Similar to FEME, CLIC/GEEC endocytosis occurs at the edge of migrating cells and involves tubular carriers. However, unlike FEME, CLIC/GEEC, endocytosis is a constitutive pathway that mediates the internalization, among others, of hyaluronic acid receptor (CD44) and glycosylphosphatidylinositol-anchored proteins, the adeno-associated virus 2 (AAV2), as well as fluids and membrane [32]. Moreover, CLICs do not pre-form on the plasma membrane before receptor activation. After pathway activation, CLICs tubules mature into GEECs and such process is modulated by ARF1/GBF1, the actin regulatory complex Arp2/3, and the small GTPase Cdc42. For their maturation, CLICs also require the binding of two specific BAR domain proteins to the membrane, IRSp53 and GRAF1, respectively [33]. In addition to the intracellular machinery, extracellular lectins called galectins also contribute to cluster CLIC cargoes on the plasma membrane prior to their invagination [34]. The CLICs pathway is highly sensitive to changes in membrane tension and can, in turn, regulate plasma membrane tension homoeostasis as well. Such regulation is coordinated by the mechano-transducer protein vinculin [35].

2.2. IL2Rβ Uptake

IL2Rβ receptor is generally internalized by FEME in T cells [36]. However, it can also internalize using a distinct, unconventional route, which involves the WAVE 1 complex [37]. Mechanistically, the recruitment of WAVE1 to the cytosolic tail of IL-2Rβ leads to IL-2Rβ clustering and N-WASP activation. N-WASP activation induces local Arp2/3-mediated actin protrusions, which generate macropinocytic-like endocytic pits. This process is further supported by an intracellular signaling involving PI3K, Rac1, and PAK-1 activation [37]. Although similar to the macropinocytosis process, IL-2Rβ endocytosis generates smaller (<0.5 µm) and confined spherical carriers.

2.3. Macropinocytosis and Phagocytosis

Macropinocytosis and phagocytosis are endocytic processes that involve the internalization of large volume fractions of liquids or large-sized particles. Even though they differ in their nature of induction and mechanisms, these processes share multiple mechanistic similarities, including slow kinetics, major membrane remodeling, and cytoskeleton support [38]. Macropinocytosis is a unique process which rapidly allows the intake of large amounts of fluids in different cell types, including immune cells, epithelial, fibroblasts, neurons, microglia, and cancer cells [39]. Although constitutively active in quiescent circulating cells, macropinocytosis is downregulated in mature immune cells [38]. Macropinosomes can vary in size (0.2 to 10 µm in diameter) and can be modulated by both pathogens and chemical compounds. As macropinocytosis is not identical in different cell types, the most common feature is the strict dependency on actin-polymerization machinery and on both Rac1 and PAK1 recruitment [39]. Despite its importance to physiology, the molecular mechanisms underlying macropinocytosis remain only partly understood. For example, how and which molecules contribute in macropinosome scission from the plasma membrane, instead, is still unknown. This is largely due to the difficulty in studying macropinosomes owing to the lack of unique molecules present in these structures.

Phagocytosis is a universal pathway that involves the uptake of large particles (>0.5 µm), including nutrients and pathogens such as bacteria. Macrophages, neutrophils, monocytes, dendritic cells, and osteoclasts are called professional phagocytes, as they perform phagocytosis with high efficiency [40]. This process requires triggered cell surface membrane deformations that usually encircle the particle, which result in phagosomes formation [41]. Two well-described types of phagocytic processes exist: (i) FcR-mediated engulfment of immunoglobulin G-opsonized particles and (ii) complement receptor CR3-mediated ingestion of C3bi-coated particles [40][41]. FcR-mediated phagocytosis, also known as ‘zipper-like’, is mediated by the binding of FcR receptors to the ligands and the activation of a local signaling response which activates actin rearrangement, membrane extension around the ligand, and finally formation of a protruding cup

with a zipper-lock arrangement around the pathogen. CR3-type or ‘trigger-like’ phagocytosis, instead, is generally activated by extracellular chemical compounds or particles, which are then loosely encased in a large membrane vesicle. In this process, actin is activated to create local patches that control cell membrane depression. Once formed, phagosomes are gradually acidified and cargo-degraded. Both phagocytic process and phagosome degradation can be manipulated by pathogens to either promote their internalization or escape their degradation [42].

3. Caveolar Endocytosis

Although controversial, another endocytic process which uses a membrane coat is caveolin. Caveolae are characterized by a unique morphology composed by a bulb-shaped pit of approximately 60–80 nm diameter connected to the plasma membrane by a slightly smaller neck [43]. Structurally, caveolae are formed by assembly of cholesterol binding membrane proteins, termed caveolins, and cytoplasmic protein termed cavins. There are three subtypes of caveolin proteins, two of which are ubiquitarian (cav-1 and cav-2) and one is muscle specific (cav-3). Caveolae formation is cholesterol-dependent and loss in membrane cholesterol leads to disassembly of the caveolar structures. Although highly abundant in some cell types, caveolae are absent in neurons and many blood cells. In cells with abundant caveolae, such as skeletal and smooth muscle, adipocytes, and endothelial cells, recent evidence suggests that caveolae mediate mechano-protection and control of lipid homeostasis, likely acting as a general stress-sensing membrane domain [44][45].

Early work focused on caveolar endocytosis for albumin, toxins such as tetanus and cholera toxin, and viruses such as polyoma and simian 40 (SV40) and identified SV40 entry in a specific endocytic structure, termed the caveosome. This compartment had a neutral pH and did not accumulate the lysosomal dye lysotracker [46]. However, more than a decade ago, new elegant evidence provided by the same group demonstrated that caveolae bud, albeit infrequently, from the plasma membrane carrying caveolin and cavins to the classical early endosome, recommending to dismiss the term caveosome [47]. More recently, caveolar endocytosis has been described as a high-efficiency route for cytosolic siRNA delivery of polymeric nanoparticles in macrophages by circumventing lysosomes [48]. Despite these new findings, endocytosis via caveolae remains a controversial issue. It is not clear, for example, whether this endocytic route is constitutive, albeit occurring at a low rate and massively up-regulated upon specific triggers. Insights have been obtained by caveolin knockout mice, which revealed novel roles of caveolin and clarify caveolar cargo specificity [49]. However, the lack of selective specificity for traditional caveolae cargoes observed in knockout mice has raised the hypothesis that caveolin expression level is critical in defining the entry route of certain cargoes. Endocytosis of SV40 virus, for example, has been reported to increase in cells lacking caveolin-1, suggesting an inhibitory role of caveolin-1 in this process [50][51].

References

1. McMahon, H.T.; Boucrot, E. Molecular Mechanism and Physiological Functions of Clathrin-Mediated Endocytosis. Nat. Rev. Mol. Cell Biol. 2011, 12, 517–533.
2. Anderson, R.G.W.; Brown, M.S.; Goldstein, J.L. Role of the Coated Endocytic Vesicle in the Uptake of Receptor-Bound Low Density Lipoprotein in Human Fibroblasts. Cell 1977, 10, 351–364.
3. Benmerah, A.; Lamaze, C. Clathrin-Coated Pits: Vive La Différence? Traffic 2007, 8, 970–982.
4. Kirchhausen, T.; Owen, D.; Harrison, S.C. Molecular Structure, Function, and Dynamics of Clathrin-Mediated Membrane Traffic. Cold Spring Harb. Perspect. Biol. 2014, 6, a016725.
5. Smith, S.M.; Baker, M.; Halebian, M.; Smith, C.J. Weak Molecular Interactions in Clathrin-Mediated Endocytosis. Front. Mol. Biosci. 2017, 4, 72.
6. Ehrlich, M.; Boll, W.; van Oijen, A.; Hariharan, R.; Chandran, K.; Nibert, M.L.; Kirchhausen, T. Endocytosis by Random Initiation and Stabilization of Clathrin-Coated Pits. Cell 2004, 118, 591–605.
7. Puthenveedu, M.A.; von Zastrow, M. Cargo Regulates Clathrin-Coated Pit Dynamics. Cell 2006, 127, 113–124.
8. Sigismund, S.; Woelk, T.; Puri, C.; Maspero, E.; Tacchetti, C.; Transidico, P.; di Fiore, P.P.; Polo, S. Clathrin-Independent Endocytosis of Ubiquitinated Cargos. Proc. Natl. Acad. Sci. USA 2005, 102, 2760–2765.
9. Watanabe, S.; Mamer, L.E.; Raychaudhuri, S.; Luvsanjav, D.; Eisen, J.; Trimbuch, T.; Söhl-Kielczynski, B.; Fenske, P.; Milosevic, I.; Rosenmund, C.; et al. Synaptojanin and Endophilin Mediate Neck Formation during Ultrafast Endocytosis. Neuron 2018, 98, 1184–1197.e6.
10. Cossart, P.; Helenius, A. Endocytosis of Viruses and Bacteria. Cold Spring Harb. Perspect. Biol. 2014, 6, a016972.
11. Mercer, J.; Schelhaas, M.; Helenius, A. Virus Entry by Endocytosis. Annu. Rev. Biochem. 2010, 79, 803–833.

12. Veiga, E.; Cossart, P. Listeria InlB Takes a Different Route to Met. Cell 2007, 130, 218–219.

13. Latomanski, E.A.; Newton, H.J. Taming the Triskelion: Bacterial Manipulation of Clathrin. Microbiol. Mol. Biol. Rev. 2019, 83, e00058-18.

14. Casamento, A.; Boucrot, E. Molecular Mechanism of Fast Endophilin-Mediated Endocytosis. Biochem. J. 2020, 477, 2327–2345.

15. Ferreira, A.P.A.; Casamento, A.; Carrillo Roas, S.; Halff, E.F.; Panambalana, J.; Subramaniam, S.; Schützenhofer, K.; Chan Wah Hak, L.; McGourty, K.; Thalassinos, K.; et al. Cdk5 and GSK3β Inhibit Fast Endophilin-Mediated Endocytosis. Nat. Commun. 2021, 12, 2424.

16. Sigismund, S.; Argenzio, E.; Tosoni, D.; Cavallaro, E.; Polo, S.; di Fiore, P.P. Clathrin-Mediated Internalization Is Essential for Sustained EGFR Signaling but Dispensable for Degradation. Dev. Cell 2008, 15, 209–219.

17. Sigismund, S.; Algisi, V.; Nappo, G.; Conte, A.; Pascolutti, R.; Cuomo, A.; Bonaldi, T.; Argenzio, E.; Verhoef, L.G.G.C.; Maspero, E.; et al. Threshold-Controlled Ubiquitination of the EGFR Directs Receptor Fate. EMBO J. 2013, 32, 2140–2157.

18. Ferreira, A.P.A.; Boucrot, E. Mechanisms of Carrier Formation during Clathrin-Independent Endocytosis. Trends Cell Biol. 2018, 28, 188–200.

19. Caldieri, G.; Barbieri, E.; Nappo, G.; Raimondi, A.; Bonora, M.; Conte, A.; Verhoef, L.G.G.C.; Confalonieri, S.; Malabarba, M.G.; Bianchi, F.; et al. Reticulon 3-Dependent ER-PM Contact Sites Control EGFR Non-Clathrin Endocytosis Europe PMC Funders Group. Science 2017, 356, 617–624.

20. Watanabe, S.; Boucrot, E. Fast and Ultrafast Endocytosis. Curr. Opin. Cell Biol. 2017, 47, 64–71.

21. Watanabe, S.; Rost, B.R.; Camacho-Pérez, M.; Davis, M.W.; Söhl-Kielczynski, B.; Rosenmund, C.; Jorgensen, E.M. Ultrafast Endocytosis at Mouse Hippocampal Synapses. Nature 2013, 504, 242–247.

22. Watanabe, S.; Trimbuch, T.; Camacho-Pérez, M.; Rost, B.R.; Brokowski, B.; Söhl-Kielczynski, B.; Felies, A.; Davis, M.W.; Rosenmund, C.; Jorgensen, E.M. Clathrin Regenerates Synaptic Vesicles from Endosomes. Nature 2014, 515, 228–233.

23. Manni, M.M.; Tiberti, M.L.; Pagnotta, S.; Barelli, H.; Gautier, R.; Antonny, B. Acyl Chain Asymmetry and Polyunsaturation of Brain Phospholipids Facilitate Membrane Vesiculation without Leakage. eLife 2018, 7, e34394.

24. Chanaday, N.L.; Cousin, M.A.; Milosevic, I.; Watanabe, S.; Morgan, J.R. The Synaptic Vesicle Cycle Revisited: New Insights into the Modes and Mechanisms. J. Neurosci. 2019, 39, 8209–8216.

25. Cheung, G.; Cousin, M.A. Synaptic Vesicle Generation from Activity-Dependent Bulk Endosomes Requires Calcium and Calcineurin. J. Neurosci. 2013, 33, 3370–3379.

26. Nicholson-Fish, J.C.; Kokotos, A.C.; Gillingwater, T.H.; Smillie, K.J.; Cousin, M.A. VAMP4 Is an Essential Cargo Molecule for Activity-Dependent Bulk Endocytosis. Neuron 2015, 88, 973–984.

27. Römer, W.; Berland, L.; Chambon, V.; Gaus, K.; Windschiegl, B.; Tenza, D.; Aly, M.R.E.; Fraisier, V.; Florent, J.C.; Perrais, D.; et al. Shiga Toxin Induces Tubular Membrane Invaginations for Its Uptake into Cells. Nature 2007, 450, 670–675.

28. Kirkham, M.; Fujita, A.; Chadda, R.; Nixon, S.J.; Kurzchalia, T.V.; Sharma, D.K.; Pagano, R.E.; Hancock, J.F.; Mayor, S.; Parton, R.G. Ultrastructural Identification of Uncoated Caveolin-Independent Early Endocytic Vehicles. J. Cell Biol. 2005, 168, 465–476.

29. Parkyn, C.J.; Vermeulen, E.G.M.; Mootoosamy, R.C.; Sunyach, C.; Jacobsen, C.; Oxvig, C.; Moestrup, S.; Liu, Q.; Bu, G.; Jen, A.; et al. LRP1 Controls Biosynthetic and Endocytic Trafficking of Neuronal Prion Protein. J. Cell Sci. 2008, 121, 773–783.

30. Sarnataro, D.; Caputo, A.; Casanova, P.; Puri, C.; Paladino, S.; Tivodar, S.S.; Campana, V.; Tacchetti, C.; Zurzolo, C. Lipid Rafts and Clathrin Cooperate in the Internalization of PrPC in Epithelial FRT Cells. PLoS ONE 2009, 4, e5829.

31. Lundmark, R.; Doherty, G.J.; Howes, M.T.; Cortese, K.; Vallis, Y.; Parton, R.G.; McMahon, H.T. The GTPase-Activating Protein GRAF1 Regulates the CLIC/GEEC Endocytic Pathway. Curr. Biol. 2008, 18, 1802–1808.

32. Howes, M.T.; Kirkham, M.; Riches, J.; Cortese, K.; Walser, P.J.; Simpson, F.; Hill, M.M.; Jones, A.; Lundmark, R.; Lindsay, M.R.; et al. Clathrin-Independent Carriers Form a High Capacity Endocytic Sorting System at the Leading Edge of Migrating Cells. J. Cell Biol. 2010, 190, 675–691.

33. Sathe, M.; Muthukrishnan, G.; Rae, J.; Disanza, A.; Thattai, M.; Scita, G.; Parton, R.G.; Mayor, S. Small GTPases and BAR Domain Proteins Regulate Branched Actin Polymerisation for Clathrin and Dynamin-Independent Endocytosis. Nat. Commun. 2018, 9, 1835.

34. Rennick, J.J.; Johnston, A.P.R.; Parton, R.G. Key Principles and Methods for Studying the Endocytosis of Biological and Nanoparticle Therapeutics. Nat. Nanotechnol. 2021, 16, 266–276.
35. Thottacherry, J.J.; Kosmalska, A.J.; Kumar, A.; Vishen, A.S.; Elosegui-Artola, A.; Pradhan, S.; Sharma, S.; Singh, P.P.; Guadamillas, M.C.; Chaudhary, N.; et al. Mechanochemical Feedback Control of Dynamin Independent Endocytosis Modulates Membrane Tension in Adherent Cells. Nat. Commun. 2018, 9, 4217.
36. Lamaze, C.; Dujeancourt, A.; Baba, T.; Lo, C.G.; Benmerah, A.; Dautry-Varsat, A. Interleukin 2 Receptors and Detergent-Resistant Membrane Domains Define a Clathrin-Independent Endocytic Pathway. Mol. Cell 2001, 7, 661–671.
37. Grassart, A.; Dujeancourt, A.; Lazarow, P.B.; Dautry-Varsat, A.; Sauvonnet, N. Clathrin-Independent Endocytosis Used by the IL-2 Receptor Is Regulated by Rac1, Pak1 and Pak2. EMBO Rep. 2008, 9, 356–362.
38. Kerr, M.C.; Teasdale, R.D. Defining Macropinocytosis. Traffic 2009, 10, 364–371.
39. Lin, X.P.; Mintern, J.D.; Gleeson, P.A. Macropinocytosis in Different Cell Types: Similarities and Differences. Membranes 2020, 10, 177.
40. Uribe-Querol, E.; Rosales, C. Phagocytosis: Our Current Understanding of a Universal Biological Process. Front. Immunol. 2020, 11, 1066.
41. Kumari, S.; Mg, S.; Mayor, S. Diversity of Endocytic Mechanisms 256 Endocytosis Unplugged: Multiple Ways to Enter the Cell. Cell Res. 2010, 20, 256–275.
42. Kinchen, J.M.; Ravichandran, K.S.S. Phagocytic Signaling: You Can Touch, but You Can’t Eat. Curr. Biol. 2008, 18, R521–R524.
43. Parton, R.G.; Tillu, V.; McMahon, K.A.; Collins, B.M. Key Phases in the Formation of Caveolae. Curr. Opin. Cell Biol. 2021, 71, 7–14.
44. Parton, R.G.; Kozlov, M.M.; Ariotti, N. Caveolae and Lipid Sorting: Shaping the Cellular Response to Stress. J. Cell Biol. 2020, 219, e201905071.
45. Lo, H.P.; Hall, T.E.; Parton, R.G. Mechanoprotection by Skeletal Muscle Caveolae. BioArchitecture 2016, 6, 22–27.
46. Pelkmans, L.; Helenius, A. Endocytosis via Caveolae. Traffic 2002, 3, 311–320.
47. Hayer, A.; Stoeber, M.; Ritz, D.; Engel, S.; Meyer, H.H.; Helenius, A. Caveolin-1 Is Ubiquitinated and Targeted to Intralumenal Vesicles in Endolysosomes for Degradation. J. Cell Biol. 2010, 191, 615–629.
48. Song, Y.; Wu, Y.; Xu, L.; Jiang, T.; Tang, C.; Yin, C. Caveolae-Mediated Endocytosis Drives Robust SiRNA Delivery of Polymeric Nanoparticles to Macrophages. ACS Nano 2021, 15, 8267–8282.
49. Damm, E.M.; Pelkmans, L.; Kartenbeck, J.; Mezzacasa, A.; Kurzchalia, T.; Helenius, A. Clathrin- and Caveolin-1-Independent Endocytosis: Entry of Simian Virus 40 into Cells Devoid of Caveolae. J. Cell Biol. 2005, 168, 477–488.
50. Renard, H.F.; Boucrot, E. Unconventional Endocytic Mechanisms. Curr. Opin. Cell Biol. 2021, 71, 120–129.
51. Xing, Y.; Wen, Z.; Gao, W.; Lin, Z.; Zhong, J.; Jiu, Y. Multifaceted Functions of Host Cell Caveolae/Caveolin-1 in Virus Infections. Viruses 2020, 12, 487.