Endocytosis: Past, Present, and Future

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Endocytosis, the process of cellular ingestion, may have been the driving force behind evolution of the eucaryotic cell (de Duve 2007). Acquiring the ability to internalize macromolecules and digest them intracellularly would have allowed primordial cells to move out from their food sources and pursue a predatory existence; one that might have led to the development of endosymbiotic relationships with mitochondria and plastids. Thus, it is fitting that endocytosis was first discovered and named as the processes of cell "eating" and "drinking." In 1883, the developmental biologist Ilya Metchnikoff coined the term phagocytosis, from the Greek "phagos" (to eat) and "cyte" (cell), after observing motile cells in transparent starfish larva surround and engulf small splinters that he had inserted (Tauber 2003). Decades later, in 1931, Warren H. Lewis, one of the earliest cell "cinematographers" coined the term pinocytosis, from the Greek "pinean" (to drink), after observing the uptake of surrounding media into large vesicles in cultured macrophages, sarcoma cells, and fibroblasts by time-lapse imaging (Lewis 1931; Corner 1967).

Importantly, these pioneering studies also revealed that the function of endocytosis goes well beyond eating and drinking. Indeed, Metchnikoff, considered one of the founders of modern immunology, realized that the phagocytic behavior of the mesodermal amoeboid cells he had observed under the microscope could serve as a general defense system against invasive parasites, in the larva as in man. This revolutionary concept, termed the phagocytic theory, earned Metchnikoff the 1908 Nobel Prize in Physiology or Medicine for his work on phagocytic immunity, which he shared with Paul Ehrlich who discovered the complementary mechanisms of humoral immunity that led to the identification of antibodies (Vaughan 1965; Tauber 2003; Schmalstieg and Goldman 2008). The phagocytic theory was a milestone in immunology and cell biology, and formally gave birth to the field of endocytosis.

Key discoveries over the intervening years, aided in large part by the advent of electron microscopy, revealed multiple pathways for endocytosis in mammalian cells that fulfill multiple critical cellular functions (Fig. 1). These mechanistically and morphologically distinct pathways, and their discoverers, include clathrin-mediated endocytosis (Roth and Porter 1964), caveolae uptake (Palade 1953; Yamada 1955), cholesterol-sensitive clathrin- and caveolae-independent pathways (Moya et al. 1985; Hansen et al. 1991; Lamaze et al. 2001), and, more recently, the large capacity CLIC/GEEC pathway (Kirkham et al. 2005). In place of Metchnikoff's splinters, many of these discoveries resulted

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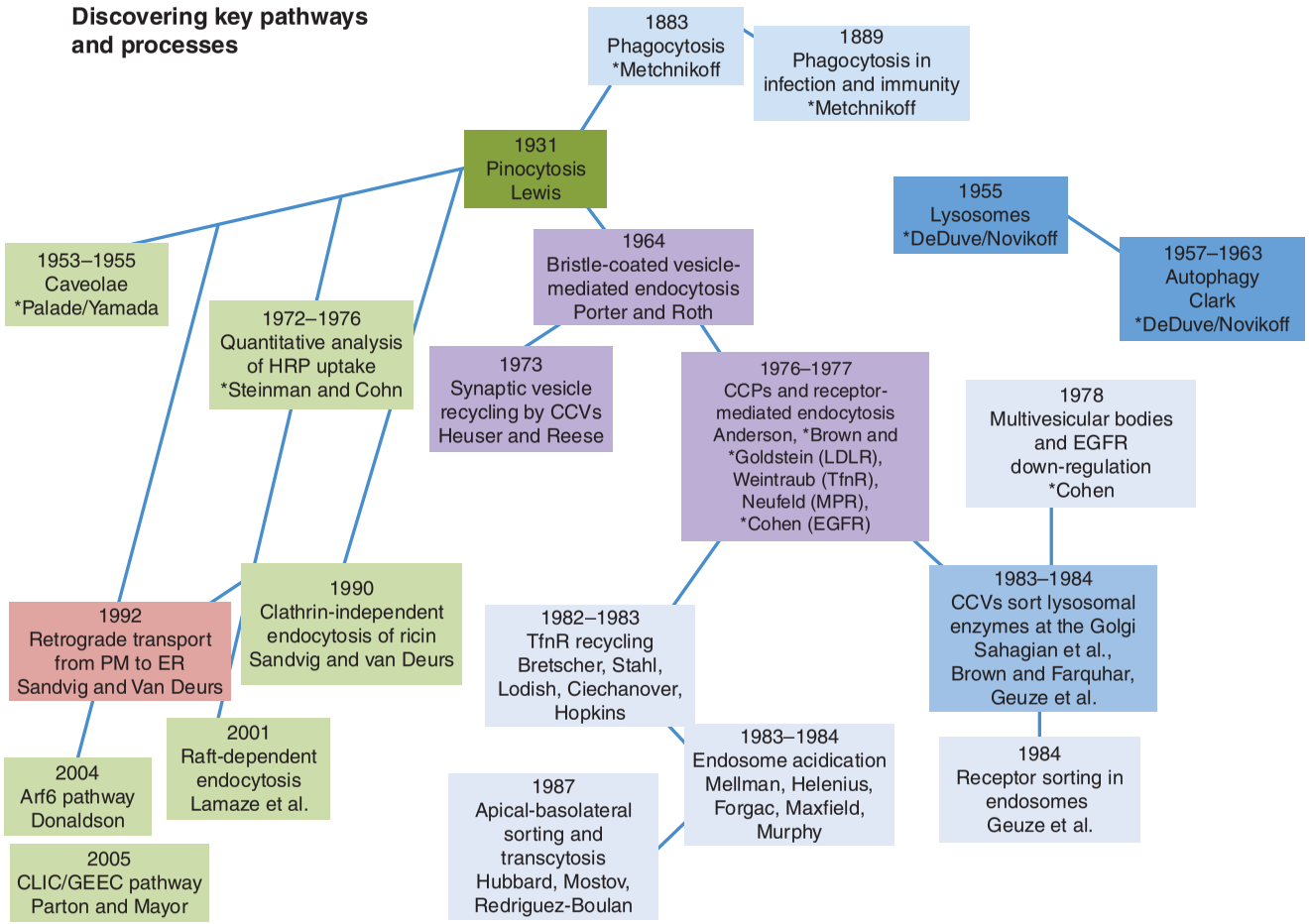

Figure 1. Time line for discoveries of endocytic pathways and their discoverers. Boxes are color-coded by pathway. *, Nobel laureate. HRP, horseradish peroxidase; CCVs, clathrin-coated vesicles; CCPs, clathrin-coated pits; EGFR, epidermal growth factor receptor; PM, plasma membrane; ER, endoplasmic reticulum; CLIC/GEEC, clathrin-independent carriers/GPI-enriched endocytic compartments.

from the detection and tracking of internalized HRP-, ferritin-, or gold-conjugated ligands by electron microscopy. These electron-dense tracers allowed researchers to identify cellular structures associated with the uptake and intracellular sorting of receptor-bound ligands. A particularly striking example is the pioneering work of Roth and Porter, who in 1964 observed the uptake of yolk proteins into mosquito oocytes. To synchronize uptake, they killed female mosquitos at timed intervals after a blood feed and observed the sequential appearance of electron-dense yolk granules in coated pits, coated and uncoated vesicles, and progressively larger vesicles. Their remarkable observations accurately described coated vesicle budding, uncoating, homo- and heterotypic fusion events, as well as the emergence of tubules from early endosomes (Fig. 2), all of which are now known hallmarks of the early endocytic trafficking events.

Another milestone in the field of endocytosis was the discovery of the lysosome by Christian de Duve (Appelmans et al. 1955). Whereas the finding of phagocytosis and other endocytic pathways was possible through microscopy, the discovery of lysosomes originated from a biochemical approach (Courtoy 2007), which benefited from the invention of the ultracentrifuge. de Duve and coworkers observed that preparations of acid phosphatase obtained by subcellular fractionation had an unusual behavior: contrary to most enzymatic activities, the activity of acid phosphatase increased rather than decreased with time, freezing–thawing of the fractions and the presence of detergents. Interestingly, the same was true for other hydrolases, which depended on acidic pH for their optimal activity. This led him to postulate that the acid hydrolases were contained in acidified membrane-bound vesicles. In collaboration with Alex Novikoff, he visualized these vesicles, the lysosomes, by electron microscopy (Beaufay et al. 1956) and later showed their content of acid phosphatase (Farquhar et al. 1972). In

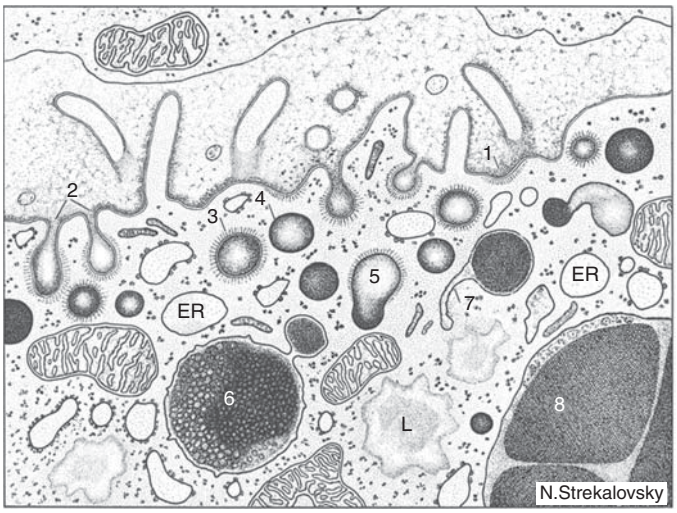

Figure 2. Fiftieth anniversary of the discovery of clathrin-mediated endocytosis by Roth and Porter (1964). The image is the hand-drawn summary of observations made by electron microscopic examination of the trafficking of yolk proteins in a mosquito oocyte. Note the many details, later confirmed and mechanistically studied over the intervening 50 years. These include the growth, invagination, and pinching off of coated pits (1,2), which concentrate cargo taken up by coated vesicles (3), the rapid uncoating of nascent-coated vesicles (4), homotypic fusion of nascent endocytic vesicles in the cell periphery (5), the formation of tubules from early endosomes (7), and changes in the intraluminal properties of larger endosomes (6). Finally, yolk proteins are stored in granules as crystalline bodies (8). (From Roth and Porter 1964; reprinted, with express permission, from Rockefeller University Press © 1964, *The Journal of Cell Biology* **20**: 313–332, doi: 10.1083/jcb.20.2.313.)

1974, de Duve was awarded the Nobel Prize for Physiology or Medicine for his seminal finding of the lysosomes and peroxisomes. He shared it with Albert Claude and George E. Palade "for their discoveries concerning the structural and functional organization of the cell." The importance of this work lies also in the significant therapeutic applications that followed. The discovery by Elizabeth Neufeld and collaborators of uptake of lysosomal enzymes by cells provided the foundation for enzyme replacement therapy for lysosomal storage disorders (Neufeld 2011).

In the 1970s, research in endocytosis entered the molecular era. Using de Duve and Albert Claude-like methods of subcellular fractionation, Barbara M. Pearse purified clathrin-coated vesicles from pig brain (Pearse 1975). A year later, she isolated a major protein species of 180 kDa, which she named clathrin "to indicate the lattice-like structures which it forms" (Pearse 1976). It was a breakthrough that inaugurated the molecular dissection of clathrin-mediated endocytosis.

Over the intervening years, the continued application of microscopy (which now spans from electron cryotomography to live cell, high-resolution fluorescence microscopy), genetics (in particular, in yeast, *Caenorhabditis elegans* and *Drosophila melanogaster*), biochemistry (including cell-free reconstitution of endocytic membrane trafficking events), as well as molecular and structural biology have revealed a great deal about the cellular machineries and mechanisms that govern trafficking along the endocytic pathway. A partial, and because of space limitations, necessarily incomplete list of milestones (Table 1) shows how new technologies (e.g., green fluorescent protein [GFP] fusion proteins, total internal reflection microscopy, siRNA knockdowns) enable new discoveries.

OVERVIEW OF ARTICLES

In organizing this collection, our intention was to summarize a great deal of established concepts and mechanisms, and also to highlight outstanding questions and controversies in the field. We first describe our current knowledge of the core molecular machinery governing endocytosis and transport along the endocytic pathway, followed by examples of the importance of endocytic trafficking in organismal physiology and development, and how defects in endocytosis or its cooptation can lead to and also benefit human disease. Each article ends with a discussion of future perspectives, including outstanding questions, challenges, and opportunities for new discovery.

The order of articles reflects broadly the spatiotemporal progression of cargo from the plasma membrane toward the lysosome. We begin with several articles that describe our current understanding of the molecular machinery and mechanisms driving entry of cargo (e.g., receptors and bound ligands) into the cell, by clathrin-dependent and -independent endocytosis (Traub and Bonifacino 2013; Kirchhausen et al. 2014; Mayor et al. 2014; Merrifield and Kaksonen 2014; Mettlen and Danuser 2014). Following internalization, the endocytosed material is shuttled along an ultrastructurally complex and dynamic pathway of tubular and vesicular compartments collectively known as endosomes, and described in the article by Klumperman and Raposo (2014), from which they can be sorted for recycling back to the cell surface or delivered to lysosomes for degradation. Several articles are then devoted to the signals and mechanisms governing these sorting decisions. They cover the molecular machineries responsible for the compartmental organization and function of endosomes (Wandinger-Ness and Zerial 2014) and sorting and transport along endolysosomal pathways (Bissig and Gruenberg 2013; Henne et al. 2013; Burd and Cullen 2014; Piper et al. 2014). Lysosomes and lysosome-related organelles are discussed in the article by Luzio et al. 2014). Once thought to be "dead-end" receptacles responsible only for degradation, lysosomes have revealed themselves as dynamic and functionally diverse organelles that can fuse with the plasma membrane in response to extracellular cues. The common biophysical challenges faced in deforming the plasma membrane or endosomes to generate transport vesicles are discussed in

Table 1. Some key discoveries in endocytic trafficking

Year	Mechanistic milestones	Discoverers
1973	Identification of shibirets (dynamin) mutant in *Drosophila*	D. Suzuki and C. Poodry
1974–1976	Zipper mechanism for phagocytosis	S. Silverstein
1975–1976	Isolation of CCVs, purification of clathrin	B. Pearse
1982–1984	Phosphomannose, M6PR, and lysosomal targeting	W. Sly, S. Kornfeld, E. Neufeld, G. Sahagian
1983–1984	Isolation of clathrin adapters/localization to distinct membranes	J. Keen, B. Pearse, M. Robinson
1986	Isolation of endocytosis mutants (End) in yeast	H. Riezman
1986–1987	Isolation of vacuolar protein sorting mutants in yeast	S. Emr, T. Stevens
1986	Endosome fusion in vitro	J. Gruenberg and K. Howell
1986	EGF and insulin receptor signaling from endosomes	J. Bergeron and B. Posner
1986	Macropinocytosis induced in stimulated cells	D. Bar-Sagi and J. Feramisco
1987	Endocytic sorting motifs (FxNPxY, YxxF)	M. Brown and J. Goldstein, I. Trowbridge, T. McGraw
1987–1989	Cloning of CHC, CLC, AP2	T. Kirchhausen, M. Robinson
1988	Isolation of biochemically distinct early and late endosomes	S. Schmid and I. Mellman
1989–1991	Clathrin-mediated endocytosis reconstituted in vitro	E. Smythe, G. Warren, S. Schmid
1990	Localization of endosomal Rab5 and Rab7	P. Chavrier, R. Parton, M. Zerial
1991	Endosome to *trans*-Golgi network (TGN) transport reconstituted in vitro	S. Pfeffer
1992	Rab5 and Rab4 as early endocytic regulators in vivo	M. Zerial, R. Parton, I. Mellman
1992–1995	Caveolin/VIP21 identified as caveolar coat protein	R. Anderson, T. Kurzchalia, R. Parton, K. Simons
1992	Vacuolar fusion reconstituted in vitro	W. Wickner
1992–1994	Trigger mechanism for phagocytosis of bacteria	S. Falkow, J. Galán, J. Swanson
1993	Actin's role in endocytosis in yeast	H. Riezman
1993	Isolation of autophagy mutants (Atg) in yeast	Y. Ohsumi
1993	PI3 kinase activity (PI3P) and endosome function	S. Emr
1993	Dynamin's role in clathrin-mediated endocytosis	R. Vallee, S. Schmid
1995	Dynamin assembles into rings	S. Schmid, P. De Camilli
1996	Clathrin-mediated endocytosis requirement for signaling	S. Schmid
1996	Long distance retrograde transport of signaling endosomes in neurons	W. Mobley
1996	PI5 phosphatase activity ($PI(4,5)P_2$) and clathrin-mediated endocytosis	P. De Camilli
1996	Ubiquitin-dependent sorting in endocytosis	R. Haguenauer-Tsapis; L. Hicke and H. Riezman
1997	AP3 and endosomal/lysosomal sorting	J. Bonifacino, S. Robinson
1998	FYVE fingers bind to PI3P	H. Stenmark
1998	LBPA in MVB biogenesis	T. Kobayashi, R. Parton, J. Gruenberg
1997–1998	Sorting nexins	G. Gill, S. Emr
1998	Structural basis for Y-based sorting signal recognition	D. Owen
1998	Retromer coat and endosome to TGN sorting	S. Emr
1998	β-Propeller structure of clathrin heavy chain terminal domain	T. Kirchhausen and S. Harrison
1998	Cargo-specific subpopulations of clathrin-coated pits	M. von Zastrow
1999	Structure of the clathrin coat protein superhelical motifs	J. Ybe and F. Brodsky
1999	Imaging green fluorescent protein–clathrin in living cells	J. Keen

Continued

Table 1. *Continued*

Year	Mechanistic milestones	Discoverers
1999	Biochemical purification of Rab5 effectors	S. Christoforidis and M. Zerial
1999	Genetic screen for endocytosis mutants in *C. elegans*	B. Grant
2000	Role of endocytosis in establishing morphogenic gradients	M. Gonzalez-Gaitan, S.M. Cohen
2000	Identification of GGA coats and lysosomal sorting	J. Bonifacino, S. Kornfeld, M. Robinson
2000	Identification of endosomal sorting complex required for transport (ESCRT) machinery for multivesicular body (MVB) formation	S. Emr
2001	Ubiquitin-dependent sorting into MVBs	R. Piper, S. Emr, H. Pelham
2002	Structure of the AP2 core	D. Owen
2003	Lipid conjugation of LC3/Atg8	Y. Ohsumi
2003–2004	siRNA studies of endocytic components	S. Robinson, E. Ungewickell, A. Sorkin
2004	BAR domains and membrane curvature generation	H. McMahon, P. De Camilli
2004	8-Å structure of a complete clathrin coat	T. Kirchhausen and S. Harrison
2005	Modular design of yeast endocytosis machinery	D. Drubin and M. Kaksonen
2005	Kinome-wide RNAi analysis of clathrin-mediated endocytosis (CME) and clathrin-independent endocytosis (CIE)	M. Zerial and L. Pelkmans
2006–2008	Reconstitution of dynamin-mediated membrane fission	A. Roux, P. De Camilli, S. Schmid, J. Zimmerberg, V. Frolov
2007	Glycosphingolipid-induced endocytosis	L. Johannes
2009	Reconstitution of Rab- and SNARE-dependent vacuolar and endosome fusion from purified components	W. Wickner, M. Zerial
2010	Cavins as major caveolae coat components	R. Parton; B. Nichols
2010	Reconstitution of ESCRT-dependent internal vesicle formation	T. Wollert and J. Hurley
2012	Reconstitution of CCV formation from minimal components	E. Ungewickell

the article by Johannes et al. (2014), and the endosome-specific machinery for fusion and fission that creates the dynamic network of membranes essential for protein sorting along this pathway is described by Gautreau et al. (2014). Recent work summarized by Settembre and Ballabio (2014) has provided insights into the transcriptional regulation of lysosome biogenesis, autophagy, and clearance. The article by Tooze et al. (2014) describes recent progress in autophagy or "self-eating," a cellular repair mechanism very important for cell homeostasis, defense mechanisms, and the prevention of a number of human diseases.

Not surprisingly, given its evolutionary importance, as suggested by de Duve, many of the proteins that drive endocytosis are highly conserved and can be traced to the earliest eukaryotic ancestors; more recent additions to the endocytic machinery are likely indicative of specialization and increased regulation of the endocytic pathways during evolution (as discussed in the article by Wideman et al. 2014). Some components of the endocytic machinery play "moonlighting" functions in divergent cellular processes (see Brodsky et al. 2014). Whether these components reflect their ancestral functions and the origins of the endocytic machinery, or new vocations, remains to be determined.

The plasma membrane is the conduit for all interactions between cells and their environment. Thus, it has become clear that, besides eating and drinking, these various endocytic pathways and subsequent endosomal sorting events play critical roles in regulating multiple aspects of cell and organismal physiology. The

next series of articles are therefore focused on the physiological functions of endocytosis. Endocytosis controls and constantly modifies the protein composition of the plasma membrane to respond to environmental changes (i.e., up- or down-regulating nutrient receptors and transporters) and is therefore essential to maintain cellular and serum homeostasis (Antonescu et al. 2014). In its specialized forms, endocytosis controls the activity and composition of neuronal synapses (Morgan et al. 2013; Cosker and Segal 2014), and immune surveillance and antigen presentation in dendritic cells (ten Broeke et al. 2013), which are critical to organismal physiology. Endocytosis of receptor tyrosine kinases and G-protein coupled receptors spatially and temporally controls their signaling activities (Di Fiore and von Zastrow 2014), whereas endocytosis of adhesion molecules and polarity markers controls cell migration and polarity. Together, these functions for endocytosis are essential for tissue morphogenesis and development (Bökel and Brand 2014; Di Fiore and von Zastrow 2014; Eaton and Martin-Belmonte 2014; Gonzalez-Gaitan and Jülicher 2014). Consequently, defects in endocytosis are linked to many human diseases (Maxfield 2014) including cancer (Mellman and Yarden 2013). Finally, many endocytic pathways are exploited by viruses, bacteria, and toxins to gain entry into the cell (Cossart and Helenius 2014), and more recently by researchers aiming to specifically and effectively deliver therapeutics for the treatment of human disease (Akinc and Battaglia 2013). Together, these articles outline the progress toward the elucidation of the molecular mechanisms of macropinocytosis and phagocytosis more than a century after Metchnikoff's pioneering microscopic observations. However, Metchnikoff used to translate his morphological observations of cells and tissues into drawings. Photomicroscopy in the 19th century was not a common method (Schmalstieg and Goldman 2008). Today, advances in light microscopy approaches allow the visualization of cells and tissues in living organisms and this practice promises a great future also in the clinics, as described by Weigert (2014) in this collection.

FUTURE PERSPECTIVES

Each article ends with a discussion of specific unanswered questions and opportunities for future research. However, collectively, we believe that the most important future challenges in endocytosis are (1) to expand our analysis beyond the current limited number of cargo molecules so as to analyze the mechanisms and physiological implications for cargo-selective regulation of endocytic trafficking; (2) to move from cell culture model systems to the study of endocytosis and endocytic trafficking in organ cultures and in vivo animal models so as to better understand the diversity of endocytic pathways and their physiological significance; and (3) to understand how endocytic trafficking and sorting processes are regulated at the cellular, tissue, and organismal levels to couple endocytosis with signaling, neurotransmission, motility, polarity, and other more complex cellular behaviors. These studies will be aided by new developments in cellular engineering, in vivo imaging, high-throughput discovery research, and computational modeling that will identify the regulatory connections that link endocytosis with other cellular processes.

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