A nostalgic look back 40 years after the discovery of receptor-mediated endocytosis

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ABSTRACT **The concept of receptor-mediated endocytosis was proposed 40 years ago in a seminal review by Joseph Goldstein, Michael Brown, and Richard Anderson. Not only their hypothesis but also the lessons learned that guided their discovery have stood the test of time. I recount some of these herein, while also looking back nostalgically at a forgotten era of scientific communication.**

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This year marks the 40th anniversary of a seminal review article published in *Nature* entitled "Coated Pits, Coated Vesicles, and Receptor-mediated Endocytosis," authored by Joe Goldstein, Mike Brown, and their collaborator, Richard Anderson (Goldstein *et al.*, 1979). Their review summarized a flurry of contemporaneous findings regarding the rapid and efficient receptor-mediated uptake of numerous protein ligands and the observation, in many cases, that internalization correlated with the concentration of receptor–ligand complexes at specialized regions of the plasma membrane demarcated by an electron-dense coat. In their review, the authors proposed four tenets that define receptor-mediated endocytosis: 1) that receptors, whose function is to bind an endogenous ligand to achieve a physiological effect, are expressed on the cell surface; 2) that ligand internalization is coupled to receptor binding; 3) that receptor-bound proteins enter through coated pits, and the receptors are either preclustered in coated pits or migrate there after ligand binding; and 4) that internalized ligands are delivered to lysosomes and degraded, although other intracellular destinations could be accessed.

No receptor had yet been purified, nor was there any information regarding the mechanisms underlying receptor-mediated endocytosis or targeting and fusion with lysosomes, yet the article proved to be "seminal." The Merriam-Webster dictionary defines "seminal" as having "contributed the seeds of later developments." Indeed, the article, published a year before I entered graduate school, inspired my own career-long pursuit of the mechanisms underlying what is now known as "clathrin-mediated endocytosis."

In rereading their review and the literature summarized in it, as well as from my conversation with Mike Brown and Joe Goldstein regarding the work, I take away many lessons and a feeling of nostalgia for how science was conducted 40 years ago. These I describe in this perspective.

The first lesson is that science often progresses in fits and starts (i.e., with much stopping and starting). Thus, although this review was published in 1979, we now credit Keith Porter and Tom Roth with the discovery, 15 years earlier, of endocytic-coated pits and vesicles (Roth and Porter, 1964). In a now-classic article—one of my all-time favorites—Roth and Porter performed a biological "pulse-chase" experiment in which they allowed female mosquitos to take a blood meal and then at various times afterwards dissected ovaries from fixed insects and examined the ultrastructure of maturing oocytes as they receive their "yolk" meal. With time, they observed electron-dense yolk material being concentrated in shallow and deeply invaginated coated pits, cytosolic-coated and partially uncoated vesicles, and then a collection of larger vesicles, many with tubules emerging from them. Their results were summarized in a final, prescient diagram of the endocytic pathway (Figure 1). The authors speculated that the pits were "*involved in selective uptake of material*" and that they pinched off and shed their coats before delivering their content to larger "*vesicular units…apparently through fusion.*" Other electron microscopic evidence of coated pits and vesicles in a variety of cells and tissues soon followed (e.g., Fawcett [1965] and Friend and Farquhar [1967]), but doubt remained. Some questioned whether the coating observed on these endocytic vesicles might be an artifact of fixation (Gray, 1972). More importantly, however compelling the static images were, without a direct link between these structures and actual measurements of the efficient uptake of macromolecules into cells, their function remained a matter of speculation.

Peaks of activity are often associated with the introduction of new methodologies, and so it goes with receptor-mediated endocytosis. Recent methodologies critical to Brown and Goldstein's discovery of receptor-mediated endocytosis were as follows: 1) the ability to culture human patient-derived fibroblasts; 2) the ability to purify and radioiodinate proteins, including low density lipoprotein (LDL) particles; and 3) the ability to conjugate proteins to iron-laden, and therefore electron-dense, ferritin. Moreover, many investigators at the National Institutes of Health, where Brown and Goldstein met

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Abbreviations used: EGF, epidermal growth factor receptor; HMG-CoA reductase, 3-hyrdroxy-3-methyl-glutaryl co-enzyme A reductase; HRP, horseradish peroxidase; LDL, low density lipoprotein.

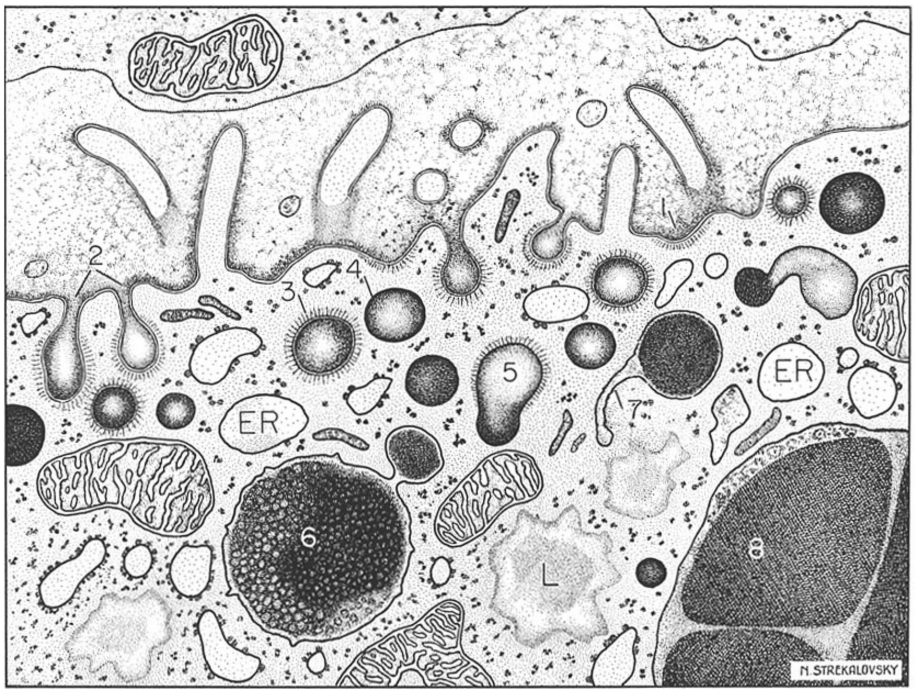

FIGURE 1: Hand-drawn summary of sequential structures (numbered 1–8) involved in uptake of yolk granule uptake in the mosquito oocyte. Reprinted from Porter and Roth, J Cell Biol, 1964.

and trained, were studying the molecular mechanisms of human genetic diseases. Brown and Goldstein's work focused on familial hypercholesterolemia and the dysregulation of cholesterol biosynthesis. Following their discovery that LDL cholesterol-trafficking particles inhibit the cholesterol biosynthetic enzyme HMG-CoA reductase (Brown *et al.*, 1974), they set out to identify the LDL receptor. They discovered that ^{125}I-labeled LDL particles bound to high-affinity, saturable, and protease-sensitive sites on the surface of normal fibroblasts but not to fibroblasts derived from patients with familial hypercholesterolemia (Brown and Goldstein, 1974; Goldstein and Brown, 1974). The bound ^{25}I-LDL was rapidly internalized and degraded in lysosomes (Goldstein *et al.*, 1975; Brown and Goldstein, 1976). As summarized in Table 1 of their review (Goldstein *et al.*, 1979), several other investigators at the time, using similar methods, reported the specific surface binding, uptake, and degradation of a number of protein ligands by various cell types, including lysosomal enzymes (Sando and Neufeld, 1977) and epidermal growth factor (Gorden *et al.*, 1978) by fibroblasts, asialoglycoproteins by hepatocytes (Ashwell and Morell, 1974), and transferrin by reticulocytes (Sullivan *et al.*, 1976).

The second lesson is the value of collaboration. Of course, their own decades-long collaboration is legendary, but at this point Brown and Goldstein teamed up with a new assistant professor at UT Southwestern, Richard Anderson, an electron microscopist. Anderson was aware of a new method to conjugate proteins to the iron-binding protein ferritin so that they could be directly visualized in electron micrographs. Anderson observed in his electron micrographs that when bound to cells at 4°C, the ferritin-conjugated LDL particles were concentrated in coated pits at varying stages of invagination. On warming to 37°C, the ferritin-LDL was rapidly taken up and delivered to lysosomes (Anderson *et al.*, 1976, 1977a). Importantly, several control experiments established the functional link between the association of LDL particles with coated pits and their uptake. Most convincing among these followed the characterization of a unique class of mutant fibroblasts that could bind but not internalize LDL. Using horseradisch peroxidase–conjugated LDL particles, Anderson showed that, in contrast to their coated pit localization in normal fibroblasts, the receptors in mutant fibroblasts were not concentrated in coated pits but were dispersed along the plasma membrane (Anderson *et al.*, 1977b).

The third lesson is the value of serendipity. Early in 1976, when Brown and Goldstein submitted their first report of the association of ferritin-LDL particles with coated pits, the question of whether these coated structures might represent a fixation artifact lingered. Their article was being considered for publication in *Proceedings of the National Academy of Sciences U.S.A.* (PNAS), and the skeptical reviewer was George Palade. Meanwhile, another distinguished scientist, Marilyn Farquhar, had reviewed a manuscript submitted three months earlier to PNAS by Barbara Pearse reporting the purification of coated vesicles from pig brain and the identification of clathrin as the major coat protein (Pearse, 1976). Pearse's findings unambiguously established clathrin-coated vesicles as *bona fide* transport vesicles. Fortunately for Brown and Goldstein, Palade and Farquhar were husband and wife; hence, the two pieces of information were immediately linked, perhaps over dinner.

The final lesson is that truly seminal articles leave as many questions unanswered as they answer. In their review, Brown and Goldstein integrated a flurry of recent studies into the new concept of receptor-mediated endocytosis, by which high-affinity cell surface receptors and their ligands are concentrated in clathrin-coated pits, specialized regions of the plasma membrane, that invaginate and pinch off to form clathrin-coated vesicles that deliver their cargo into the cell (Figure 2). The authors speculated, on the basis of their finding of a single mutant allele of LDL receptor that retained the ability to bind ligand but failed to be concentrated in coated pits and therefore to mediate LDL uptake, that surface receptors must bear

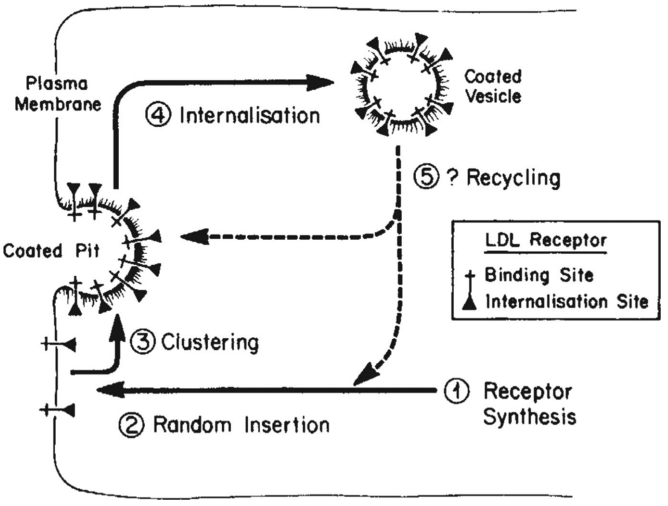

FIGURE 2: Model for receptor-mediated endocytosis from Goldstein *et al.*, 1979.

binding sites on their cytoplasmic domains that interact with clathrin or another component of the protein coat. Many questions remained. If LDL is rapidly delivered to and degraded in lysosomes, then how is it that the receptor is recycled and reutilized? What is the identity of the LDL receptor and how is it targeted to coated pits? What components of the clathrin coat function to cluster receptors? What drives coated pit invagination and the release of coated vesicles? How are they uncoated? Not all ligands are delivered to lysosomes—some traverse polarized cells, whereas others are recycled or delivered to the Golgi—so how are these sorted and targeted to the correct intracellular destination?

Indeed, several years later, at the 1982 Lysosomes Gordon Research Conference, whether coated vesicles existed remained a hotly debated issue. As a solution to the recycling problem, Ira Pastan and his coworkers argued that the pits did not pinch off. Instead, they proposed that coated pits and receptors remained at the cell surface while their ligands were deposited into uncoated membrane sacs, called "receptosomes," that emerged from their edges (Willingham and Pastan, 1980). From the audience Tom Roth shouted, "*I've studied coated vesicles my entire career, how can you say they're an artifact*?!" Alex Novikoff stood and shouted, "*Your micrographs are too poor to draw any conclusions!*" I sat quietly, fearfully awaiting the presentation of my poster on an in vitro assay to measure uncoating of these potentially artifactual entities.

The seeds planted by this seminal article grew, over the next four decades, to reveal the nature of sorting motifs, their recognition by adaptors, the existence of a complex endosomal network (sorting endosomes, recycling endosomes, multivesicular bodies), and the sorting and trafficking machinery that governs endocytic trafficking. Forty years later, while much has been learned, much remains to be learned. We still do not fully understand sorting and trafficking along the endocytic pathway, most especially how it is regulated. Moreover, the role of clathrin-mediated endocytosis has expanded from simple macromolecular uptake to the regulation of signaling from surface receptor tyrosine kinases and G protein–coupled receptors (Di Fiore and von Zastrow, 2014; Schmid, 2017; Mettlen *et al*., 2018). The link between endocytosis and signaling is a rich garden still being tended.

And now to finish with some nostalgia. The work I have described by Brown, Goldstein, and their colleagues was published in a series of articles, each reporting a single new finding, usually documented by only a few, single-panel figures. Moreover, they were communicated rapidly (Brown and Goldstein typically published an article a month) so that others could build from their findings and, importantly, put them to the test. This lively and nearly concurrent exchange of data and ideas is suppressed today by the editor-, reviewer-, and/or self-imposed constraint to publish "complete" stories. In many cases, essential data are relegated to supplemental material and often less rigorously reviewed and less frequently viewed. Editors and reviewers ask for "definitive" results, even though we know that science is in constant flux and that new techniques and perspectives can reveal new and different answers to old questions or alter interpretations of previous experiments. Most importantly, seminal articles should raise more questions than they answer! Moreover, to prepare a "complete" story, investigators tend to follow a linear path seeking additional data to "support" their hypotheses rather than more divergent paths aimed at critically "testing" them. These factors, I believe, can slow the pace of discovery and potentially lead to an increased error rate.

The articles published in the era of the print journal, when supplemental material did not exist and figures had to be visible without being enlarged, were easily digestible as separate "meals." Visit PubMed or glance at the reference list. Reading through the titles of these articles reveals the history and process of the stepwise development of a truly complete, eventually Nobel Prize–winning story. Call me old-fashioned, but I still believe in the merit of publishing a series of articles that propose and then rigorously test and modify new concepts to eventually reveal new insights into a complex cellular process. Not only is it less painful and faster, but, cumulatively, these series are often more effective, more rigorous, and, ultimately, more impactful.

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