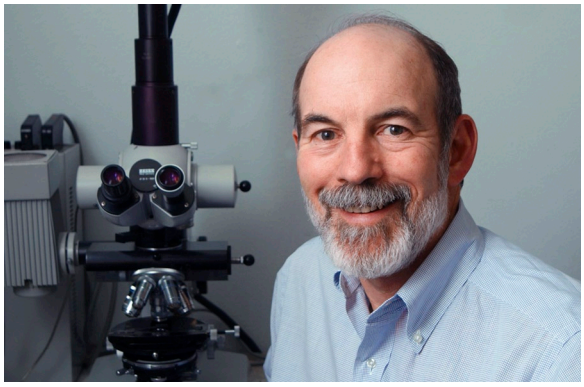
Richard G.W. Anderson (1940–2011) and the birth of receptor-mediated endocytosis

On March 19, 2011, the discipline of cell biology lost a creative force with the passing of Richard G.W. Anderson, Professor and Chairman of the Department of Cell Biology at the University of Texas Southwestern Medical School. An unabashed chauvinist for cell biology, Dick served for many years on the editorial board of *The Journal of Cell Biology* and the Council of the American Society for Cell Biology. He died of glioblastoma multiforme six days before his 71st birthday.



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Richard Anderson in 2007

Dick is responsible for two discoveries that changed our view of cell physiology: (1) receptor-mediated endocytosis in coated pits and coated vesicles (Anderson et al., 1976; Anderson et al., 1977a,b); and (2) identification of caveolin, the protein that lines the surface of caveolae (Rothberg et al., 1992). We were eye witnesses to Dick's first discovery, and here we wish to tell the tale.

The story of receptor-mediated endocytosis begins in 1973 when the two of us discovered that cultured fibroblasts from normal humans supply themselves with cholesterol through the action of a cell surface receptor that binds cholesterol-carrying low density lipoprotein (LDL), a constituent of the fetal calf serum in which cells are grown. The importance of LDL receptors was evidenced by our finding that functional receptors are absent from cells of subjects with the homozygous form of familial hypercholesterolemia (FH), a genetic disorder manifest by marked elevation in plasma LDL cholesterol and coronary heart disease in childhood. Our biochemical studies with ^{125}I-labeled LDL indicated that all of the receptor-bound LDL entered the cell within 15 minutes. Clearly, the cell could not have internalized all of its plasma membrane in this short time, suggesting that the receptors must be located in specialized regions of the plasma membrane that are adapted for rapid internalization. The challenge was to identify the mechanism by which a cell could internalize all of its receptors within 15 min. Here, we needed the culture and techniques of cell biology, a discipline that was totally new to us.

At the time, we were junior faculty members in the medicine department. A senior member of our cell biology department told us that a young faculty member named Dick Anderson had just joined his department after completing his postdoctoral studies. Dick had the knowledge and skills that we needed. He had grown up in suburban Philadelphia and had obtained a bachelor's degree in mathematics from Oregon State University and a PhD in anatomy from the University of Oregon. After a postdoctoral fellowship in which he had studied the structure of oviduct cilia by electron microscopy (EM), Dick was recruited to Dallas as a starting Assistant Professor.

We presented our problem to Dick and were overjoyed when he agreed to introduce us to cell biology. To visualize LDL by EM, Dick recommended that we crosslink LDL to the iron-containing protein ferritin. We incubated normal and FH cells with varying concentrations of LDL-ferritin at 4°C, a temperature at which our biochemical studies indicated that receptor binding occurred but internalization was prevented. We fixed the cells and gave them to Dick in a blinded fashion. Dick could easily distinguish the normal cells from the FH cells: normal cells bound LDL-ferritin at the surface, whereas the FH cells did not. What's more, when we warmed the normal cells to 37°C, Dick observed that the bound LDL entered the cells in vesicles. But that was not all that Dick saw. He also told us that the bound LDL was not dispersed at random on the cell surface. Rather, it was concentrated in regions where the membrane was indented and coated on its cytoplasmic surface by a fuzzy coat. These coated regions occupy less than 1.5% of the linear surface of the cell membrane, but they contained 70% of the bound LDL-ferritin. When the cells were warmed, the coated membrane regions invaginated and pinched off to form coated vesicles that carried the receptor-bound LDL into the cell.

In the basement of our building, we leaned excitedly over Dick's shoulders as he examined the cell surface with his electron microscope. We had never heard of coated membranes but were fascinated by Dick's findings. We soon learned that indented, coated regions of the plasma membrane had been observed by others and had been called "coated pits." Ten years earlier, pioneering studies by Roth and Porter (1964) had shown that these pits are sites where yolk proteins are taken up by mosquito oocytes during ovulation in a process that they called absorptive endocytosis. Roth and Porter showed that these pits pinch off from the plasma membrane to form coated vesicles that carry the yolk proteins into the cell.

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In retrospect, it is not surprising that Roth and Porter never postulated that a receptor mediates this absorptive process, for at that time receptors were only theoretical concepts.

To confirm that we were observing the receptor-bound LDL-ferritin, we incubated normal and FH fibroblasts with varying concentrations of LDL-ferritin. Dick worked out methods to quantify the number of ferritins per millimeter of cell surface. He found that the number of particles reached a maximum at a certain concentration of LDL-ferritin. Our biochemical studies had shown that the receptor was saturable and the binding reached a maximum at a certain concentration of ^{125}I-LDL. To convince ourselves that the electron microscope was visualizing the same process that we had detected biochemically, we labeled the LDL-ferritin preparation with ^{125}I and incubated duplicate dishes of normal and FH cells with varying concentrations of ^{125}I-labeled LDL-ferritin. One set of dishes was harvested for quantification of ^{125}I-LDL binding using scintillation counting; the duplicates were fixed and examined by Dick in the EM. Laboriously, he counted the bound ferritins. The task was especially challenging because of the rarity of coated pits. When we compared Dick's data and ours, we found a perfect correlation. The number of bound LDL-ferritins rose in proportion to the amount of ^{125}I-LDL-ferritin detected biochemically. Even though the normal and FH cells contained the same number of coated pits, the coated pits in FH cells showed no LDL-ferritin particles. We were convinced that Dick was seeing receptor-bound LDL clustered in coated pits. Two of Dick's early electron micrographs are shown in Fig. 1.

At the end of 1975, the three of us wrote a joint paper describing our findings, and we asked Earl Stadtman to communicate it to the *Proceedings of the National Academy of Sciences*. Stadtman was the premiere biochemist at the National Institutes of Health. He had been the postdoctoral advisor of one of us (M.S. Brown) and had earlier communicated two of our biochemical papers. We held him in the highest regard. After a few weeks, Earl called us and said, "Boys, I'm afraid I have bad news. I sent the paper to two reviewers. One of them thought the paper was fine. The other one said that coated pits are a well-known artifact and the paper should be rejected." We were terribly embarrassed. Earl was our hero, and we were mortified that we had asked him to endorse a paper based on an artifact. At the same time, we consulted informally with the few cell biologists whom we knew. We got mixed opinions. Some said that coated pits were real, and others told us that they were artifacts of fixation or staining. Indeed, one prominent cell biologist referred us to a paper by a British electron microscopist who stated that the coats represented an artifact of protein condensation during fixation and staining (Gray, 1972). In his characteristically calm manner, Dick was not deterred by this skepticism. He returned to his electron microscope and produced hundreds of *unstained* sections that showed unequivocally that LDL-ferritin bound to indented regions that corresponded to coated pits.

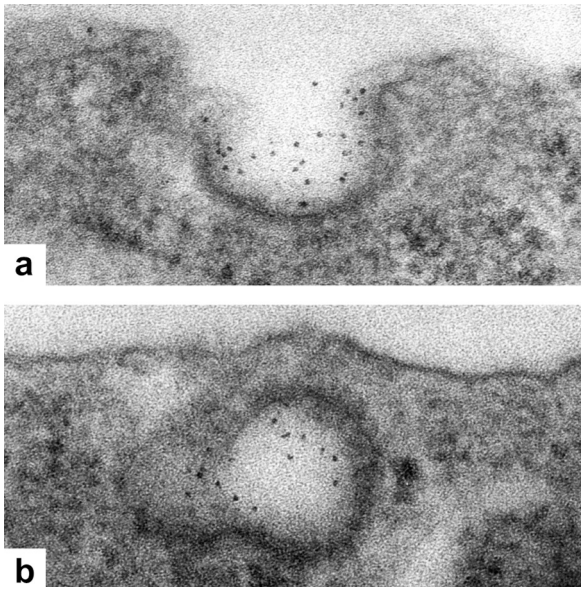

Figure 1. **Anderson's first sighting of LDL receptors in human fibroblasts.** Electron micrographs showing LDL-ferritin (black dots) in a coated pit on the cell surface (a) and in a coated vesicle inside the cell (b). These photographs were taken by Anderson in the fall of 1975. Reprinted from Anderson et al. (1977a) with permission from Elsevier.

The correlation between Dick's quantitative EM data and our biochemical data was so convincing that we concluded that he must be correct. So we asked Stadtman to seek the opinion of a third reviewer. After a few weeks, Earl called us with good news. The third reviewer recommended acceptance of the paper, and so it was rapidly published (Anderson et al., 1976). We followed this paper with another that showed the direct delivery of LDL-ferritin from coated pits to coated vesicles as cells were warmed from 4°C to 37°C (Anderson et al., 1977a). To seal the conclusion, we examined cells from a unique FH patient called J.D. In contrast to all of the FH patients that we had studied previously, J.D.'s cells bound ^{125}I-LDL, but they failed to internalize it. Dick found that the receptors in J.D.'s cells failed to localize in coated pits (Anderson et al., 1977b). This paper proved that clustering in coated pits is a prerequisite for receptor-mediated endocytosis. Together, these three papers inaugurated the field of receptor-mediated endocytosis in coated pits. Within several years, many other receptors—including those for transferrin, EGF, α-2-macroglobulin, and maternal immunoglobulins—were found to carry their ligands into cells by the same mechanism. In 1979 the three of us summarized the accumulating data in a widely cited review entitled "Coated Pits, Coated Vesicles, and Receptor-Mediated Endocytosis" (Goldstein et al., 1979).

Many years later, we learned that we had benefitted from one of the lucky coincidences that are requisite for any successful scientific career. It turns out that the third and decisive reviewer of our initial *PNAS* paper was George Palade, a founder of modern cell biology. In subsequent years, George told us that he harbored some skepticism about coated pits. However, in 1967 his wife, the noted cell biologist Marilyn Farquhar, had published an important paper describing the uptake of horseradish peroxidase via coated pits and coated vesicles in rat vas deferens (Friend and Farquhar, 1967). Moreover, through sheer

luck, only a few months before Palade received our paper for review, Farquhar had been asked by Fred Sanger to review a *PNAS* submission from Barbara Pearse, a scientist at the MRC in Cambridge, England. Pearse had purified coated vesicles and identified the protein that formed the coat, which she named clathrin (Pearse, 1976). Clearly, coated pits and vesicles were real structures that contained a unique protein. Dick's findings could now be believed, and Palade recommended that our paper be published in *PNAS*.

If George Palade and Marilyn Farquhar had not been married to each other and if Farquhar had not been a believer in coated pits, it is entirely possible that our *PNAS* paper would have been rejected. If this had occurred, would we have continued our collaboration with Dick Anderson? As novices to cell biology, would we have had the conviction to persist in collaboration with an unknown cell biologist in the face of rejection by the cell biology establishment? The world's appreciation of receptor-mediated endocytosis in coated pits and vesicles is attributable to Dick Anderson and his confidence in the structures that he saw in his electron microscope.

Our intimate collaboration with Dick lasted for 20 years (1974–1994) and resulted in 45 joint publications, including 10 in *The Journal of Cell Biology*. Independently of us, Dick made many contributions to cell biology, most notably his discovery and naming of caveolin, the protein that forms the coat of caveolae (Rothberg et al., 1992). Dick also was instrumental in the demonstration that signaling receptors and G proteins cluster in caveolae, leading him to propose that caveolae are specialized for the initiation of signal transduction events (Anderson, 1993; Chang et al., 1994).

In addition to his role in scientific discovery, Dick had a long and distinguished career as a leader of our medical school. He trained numerous graduate students and postdoctoral fellows. He served for 12 years as chairman of our Department of Cell Biology. Even after we ceased publishing jointly, Dick continued to attend our departmental works-in-progress meetings where his advice was always helpful, and often crucial.

In 1985 we received the Nobel Prize in Physiology or Medicine "for contributions concerning the regulation of cholesterol metabolism." We invited Dick and his wife Barbara to accompany us to Stockholm. When we returned to Dallas, we gave Dick a portion of our prize money. It is a remarkable coincidence that Rupert Billingham, Dick's predecessor as Chairman of Cell Biology in Dallas, also received a share of Nobel Prize money in recognition of his contribution to the Nobel Prize–winning work of Peter Medawar. Although the Nobel Committee did not include Billingham, Medawar expressed his gratitude by sharing his prize money. We felt the same about Dick Anderson. Already, we miss him.

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