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Coated pits bring in the yolk

In the early 1960s, while analyzing electron micrographs of mosquito oocytes in search of hints about chromosomal structure, Thomas Roth's roving eye became more interested in the cell surface, which was covered with pit structures lined by a cytoplasmic coat of "bristles." When he showed the find to his

The schematic model figure in the paper has remarkably withstood the test of time even as others went on to describe other types of coated vesicles seen in the cell (Friend and Farquhar, 1967), the molecular mechanism of receptor-mediated endocytosis (Anderson et al., 1977), and the clathrin coat itself (Kanaseki and Kadota,

That unwritten speculation would eventually be eloquently demonstrated by Anderson, Brown, Goldstein, and colleagues. They observed the uptake of low-density lipoprotein by receptor-mediated endocytosis (Anderson et al., 1977) and found that a familial disease characterized by high cholesterol resulted from a receptor mutation blocking internalization (Davis et al., 1986). **JCB**

A study of yolk protein uptake leads Thomas Roth and Keith Porter to propose that endocytosis is specific to a particular cargo, and the vesicle coat might be functioning in both selection and mechanical molding.

graduate advisor, Keith Porter, he noted that such structures had been seen in one or two other cell types but remained a mystery in those early years of EM. Indeed as Roth scoured other images in the lab and the literature, he saw the fuzzy-bordered pits in every cell type, "but nowhere had it been so in-your-face as it was for the oocyte," he recalls.

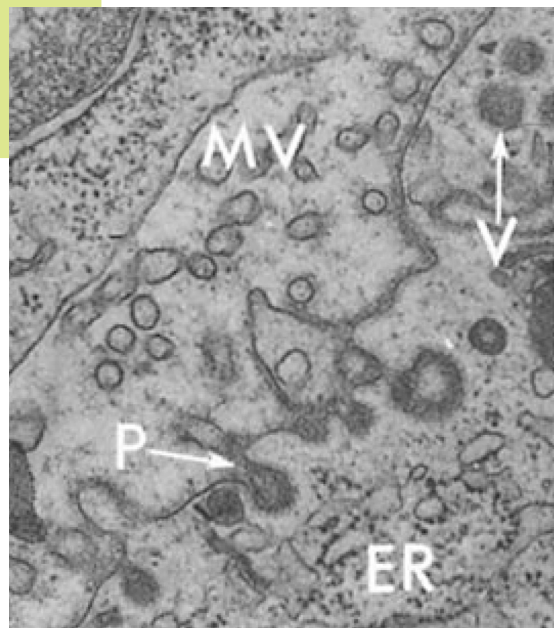
Armed with only one brief reference to "concavities" in liver cells (Fawcett, 1955) and a key paper showing, by fluorescence microscopy, that moth oocytes took up yolk protein from the extracellular space (Telfer, 1961), Roth speculated that the exaggerated yolk protein uptake going on in the oocytes must be related to the exaggerated appearance of the pits all along the cell surface. He went on to describe how the coated pits filled with yolk protein and pinched off into the cell as coated vesicles. Following the dense yolk contents, he observed the vesicles losing their bristle coats (now known as clathrin) and fusing with one another to form yolk protein bodies (Roth and Porter, 1964).

"It was germinal in setting in motion the idea of highly specific endocytosis, now known as receptor-mediated endocytosis," says Roth (University of Maryland, Baltimore, MD). "It suggested that specificity was involved in uptake [of proteins]."

1969; Pearse, 1976).

But the authors' speculations that the coat "may have a mechanical function, giving...the spherical form to the base of the pit and the pit vesicles," or determine "the specificity of materials absorbed," are perhaps the most insightful. Scottie Robinson (University of Cambridge, UK) notes that their speculations were "spot on" as we now know that vesicle coats of all types exhibit both a mechanical and selective function. She marvels that Roth, Porter, and their contemporaries made such intuitive discoveries about the endocytic and secretory pathways from static EM images. "I gather Keith Porter had this extraordinary feel for how the cell did things," she says.

But Roth still rues the paragraph that Porter made him remove from the manuscript for being overly speculative. "I was so damn mad because I had spent a fair amount of time putting together a few sentences about the various disease states and cell regulation where [uptake] might be playing a role," he remembers.



Visualization of coated pits (P) and vesicles (V) suggested that endocytosis was specific.

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Sugars sprinkled onto proteins in the Golgi

Choose the right experimental system to test a hypothesis: that has and always will be one of the ten commandments of cell biology. By the mid-1960s, several secretory cell types—liver, pancreatic, pituitary—had been exploited for tracking the path and fate of their abundant loads of secreted proteins. And by 1964, these cells had revealed a sketch of the secretory pathway from the ER to the Golgi, to vesicles, and out to the cellular membrane. This was most clearly seen when Caro and Palade (1964) combined autoradiography and electron microscopy to trace radioactive leucine in pancreatic cells.

But carbohydrates were not part of the picture, at least partly because the secretion systems studied up to that point pumped out poorly glycosylated proteins. So Marian Neutra (now at Harvard Medical School, Boston, MA) and C.P. Leblond (McGill University, Montreal, Canada) switched to goblet cells of the rat intestine, which had the twin virtues of secreting lots of carbohydrate-loaded mucin glycoprotein and having a large, distinctive U-shaped Golgi complex that was easily visualized by both light and electron microscopy. Leblond had a hunch that the addition of complex carbohydrates to proteins might occur in the Golgi, recalls Neutra, his graduate student at the time. “He suspected glycosylation might occur in the Golgi complex since rough ER was non-reactive with stains for carbohydrates, whereas in some cell types the Golgi region was reactive,” she says.

After injecting the rats with tritiated glucose, Neutra and Leblond (1966a) saw the radioactive sugars concentrating exclusively in the Golgi at early time points and then moving out to the mucigen granules later. The results were confirmed

with other sugars and other cell types (Neutra and Leblond, 1966b). “The results were striking,” says Neutra, and led to the conclusion that the Golgi is “the site where simple sugars become immobilized on larger molecules.”

Neutra says the discovery that glycoproteins that remain associated with the cell surface are also glycosylated in the Golgi was an “unexpected” bit of luck arising from the study of goblet cells in the intestinal epithelium. In the micrographs, she saw that the columnar cells next to the goblet cells were also con-

Glycosylation occurs in the Golgi complex, based on labeling with tritiated glucose carried out by Marian Neutra and C.P. Leblond.

centrating the radioactive sugars in the Golgi. At later time points the columnar cells showed a border of glycoprotein on their apical cell surfaces. “By examining a wide variety of tissues and organs, we were able to show for the first time that glycosylation probably occurs in the Golgi in all cells,” she says.

The results nicely matched the recent findings that the Golgi was the site of another protein modification, sulfation (Lane et al., 1964; Godman and Lane, 1964). Follow-up work included the demonstration of a 40-fold enrichment of galactosyl transferase activity in isolated Golgi membranes, confirming that glycosylating enzymes were in the right place to do the job (Fleischer et al., 1969). As additional radioactive sugars such as mannose and fucose became available, Leblond’s laboratory showed that there was a division of labor between cellular compartments. Mannose, the group found, is added to thyroglobulin in the ER, and then galactose and fucose are added subsequently in the Golgi complex (Whur et al., 1969; Haddad et al., 1971).

Neutra says her graduate studies of the intestinal epithelium led her to a research career focused on mucosal immunology and microbial–epithelial interactions in the intestine. But she cherishes being a part of “an extremely fun time in cell biology”—a time that was fun, “partly because of the technical limitations,” she says. “You couldn’t design recombinant molecules. The wonderful thing at that time was the beauty of the intracellular structures still being discovered.” **JCB**

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NEUTRA

Labeling of the distinctive Golgi in goblet cells identifies it as the site of glycosylation.