

# The secretory pathway at 50: a golden anniversary for some momentous grains of silver

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**ABSTRACT** The secretory pathway along which newly synthesized secretory and membrane proteins traffic through the cell was revealed in two articles published 50 years ago. This discovery was the culmination of decades of effort to unite the power of biochemical and morphological methodologies in order to elucidate the dynamic nature of the cell's biosynthetic machinery. The secretory pathway remains a central paradigm of modern cell biology. Its elucidation 50 years ago inspired tremendous multidisciplinary and on-going efforts to understand the machinery that makes it run, the adaptations that permit it to serve the needs of specialized cell types, and the pathological consequences that arise when it is perturbed.

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## HISTORICAL BACKGROUND

Speaking at a meeting of the Society of General Physiologists in 1958, George Palade referred to what he called the Heidenhain hypothesis. Rudolph Heidenhain, a German physiologist, proposed in 1875 that large vesicles in the apical cytoplasm of pancreatic exocrine cells, which he called zymogen granules, were the intracellular storage sites of digestive enzymes (Palade, 1959). Palade claimed in his presentation that no further progress had been made on understanding the secretory process since that time because of the limitations of light microscopy and biochemical analysis of intracellular events. No progress, that is, until Palade reported early results of his experiments using electron microscopy and cell fractionation.

The stage had been set for a new assault on secretion many years before. In the late 1930s, Albert Claude, a cancer investigator at the Rockefeller Institute, began to use centrifugation to separate different-sized particles from broken cells. Although Claude was not the first to try this, his approach was particularly rigorous because he carefully accounted for enzyme activities in the initial homogenate and each cell fraction (Claude, 1941, 1946a,b). This "balance sheet" (Hogeboom, 1951) enabled him to assign particular enzymes and consequently specific functions to different parts of the cell. In 1945, Claude and coworkers managed to use electron microscopy to open up what had been called the "optically empty ground substance" of the cell to exploration (Porter et al., 1945). Cell

fractionation and electron microscopy provided for the first time the tools necessary to move past Heidenhain's 19th century findings. However, Claude's innovations were not purely technical. As Claude stated in his Harvey Lecture in 1948, delivered just before his departure from Rockefeller, "it would be difficult to separate the biochemical work from the morphological observations since the microscope has constantly served as a guide or check for the chemical and biochemical studies" (Claude, 1948). Claude's biggest achievement was figuring out how to combine microscopy, cell fractionation, and biochemical analysis to determine how the cell worked.

## APPLYING THE POWER OF THE PULSE-CHASE PROTOCOL

Palade arrived at Rockefeller just after World War II to work with Claude and Keith Porter. He quickly mastered both electron microscopy and cell fractionation and made a number of advances, including discovery of the ribosome and both morphological and biochemical characterization of the endoplasmic reticulum, a new organelle first observed by Porter (Palade, 1955; Palade and Siekevitz, 1956). For the latter, Palade was joined in 1954 by Philip Siekevitz, a biochemist with experience in the use of radioactive isotopes to study protein synthesis (Siekevitz, 1952). In 1956, they published an article entitled "Liver microsomes: an integrated morphological and biochemical study," which was both a refinement of Claude's basic approach and a model for subsequent work on the secretory pathway (Palade and Siekevitz, 1956). This work began to establish the endoplasmic reticulum as the site where secretory proteins are first sequestered after their synthesis but before transport out of the cell. The liver, however, was not the best tissue in which to study secretion because of its complex structure and lack of obvious zymogen granules or well-defined secretory products. Instead, Palade and Siekevitz turned to the exocrine pancreas, the source of Heidenhain's observations (Siekevitz and Palade, 1958a–c).

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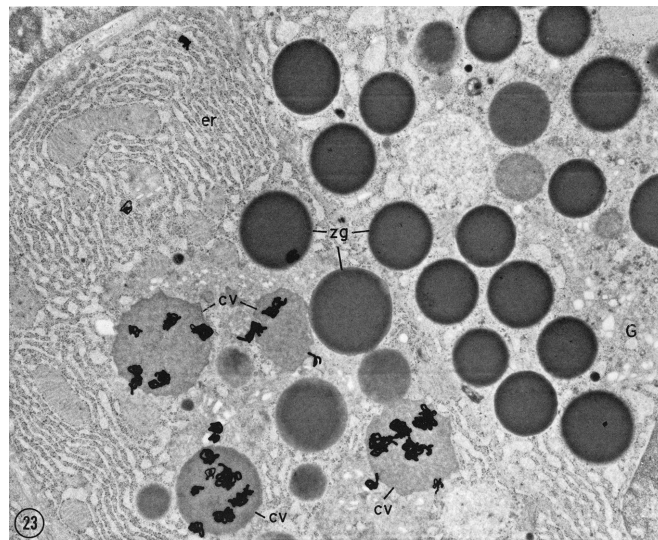
Using electron microscopy, they established that the exocrine pancreatic cells in the guinea pig contained not only zymogen granules but also abundant rough endoplasmic reticulum. After refining their cell fractionation procedures, they began to localize the digestive enzyme chymotrypsinogen to various fractions. This was laborious and slow because minuscule amounts of the enzyme had to be purified from each cell fraction using traditional biochemical procedures. Even with this effort, their approach only told them where in the cell the enzyme was concentrated but nothing about the dynamic process of secretion. Fortunately, another advantage of exocrine pancreas cells was that almost all protein synthesis was devoted to production of secretory proteins. Siekevitz and Palade capitalized on this by injecting guinea pigs with a large dose of radioactive leucine and then isolating cell fractions and purifying chymotrypsinogen from different animals at various times after injection (Siekevitz and Palade, 1958a). In this manner, they were able to demonstrate that radioactive enzyme first appeared in the rough microsomal fraction (endoplasmic reticulum) and, over a period of 15–45 min, became concentrated in the zymogen granule fraction. With this, the outline of the secretory pathway was established.

There was one problem, however. They had isolated radioactive chymotrypsinogen from cell fractions but had not definitely shown which cellular compartments contained the enzyme. Although confident about the beginning and end of the transport process, they could not confirm intermediate stages involving, for example, the Golgi complex that they had hypothesized to lie on the pathway. Fortunately, Lucien Caro, who had adapted the technique of autoradiography to electron microscopy, joined the project (Caro, 1961). Now it was possible to expose thin sections prepared from radioactively labeled pancreas to a photographic emulsion and show that, when developed, silver grains were superimposed over cellular organelles in the intact tissue that contained radioactive secretory proteins (Caro and Palade, 1964). Now the Golgi, as well as some apparently immature secretory granules, was established as an intermediate way station on the secretory pathway.

One more critical innovation was required to fully define the pathway. In injected guinea pigs, uptake of radioactive amino acids into the pancreas was both slow and asynchronous. Consequently the time resolution of these “pulse labels” was poor, particularly considering that proteins moved from their site of synthesis to the zymogen granules in ~1 h. Jim Jamieson, who joined the laboratory as a graduate student at about the time of Caro’s study, solved this problem by devising a way to incubate and radioactively label isolated pancreas slices *in vitro*. Not only was the time resolution improved, but in addition the sensitivity of the technique was much greater because higher specific activities of labeling were achieved. Using this approach combined with the previous techniques of cell fractionation and autoradiography, Jamieson and Palade definitively established, in two fundamental 1967 articles, that secretory proteins move in the exocrine pancreas from their site of synthesis in the rough endoplasmic reticulum, through peripheral elements of the Golgi complex to what they termed condensing vacuoles, before concentrating in zymogen granules before secretion (Jamieson and Palade, 1967a,b; Palade, 1975; Figure 1). Of equal importance, Jamieson’s experimental system now permitted him to perturb the secretory process and take the first steps toward mechanistic understanding (Jamieson and Palade, 1968a,b, 1971).

### THE NEXT 50 YEARS: HOW DOES THE SECRETORY PATHWAY WORK AND WHAT DOES IT DO?

Jamieson and Palade’s 1967 articles sparked the emergence of an entire subdiscipline of cell biology that has dedicated itself over the



**FIGURE 1:** Autoradiogram of a pancreatic exocrine cell pulse-labeled with radioactive leucine and then chased for 20 min. The dark silver grains over the condensing vacuoles (cv) and lack of grains over the endoplasmic reticulum (er), Golgi complex (G), or the zymogen granules (zg) indicate that secretory protein transport had progressed beyond the endoplasmic reticulum and Golgi to the condensing vacuoles but had not yet reached the zymogen granules, the final step before secretion from the cell.  $\times 16,000$ . © Marilyn G. Farquhar and George E. Palade. Originally published in Farquhar and Palade (1987), reproducing in part Figure 12 from Jamieson and Palade (1967b).

ensuing 50 years to establishing the secretory pathway’s generality and variations and to illuminating the molecular mechanisms that govern it. By visualizing the way-stations of the secretory pathway, Jamieson and Palade established that its component organelles communicate and can share content with one another, as evidenced by the stepwise and vectorial movement of newly synthesized protein cargoes. It remained to be determined how that content moves between organelles, what happens to that cargo on its journey, and how the organelles of the secretory pathway retain their structural and compositional identities in the face of this communication. A significant fraction of what we know of the molecular machinery involved in these processes was learned through the use of *in vitro* reconstitution approaches pioneered by Günter Blobel (who received the Nobel Prize in 1999) to study secretory protein translocation and segregation in the endoplasmic reticulum (Blobel and Dobberstein, 1975a,b; Blobel, 2000). These were then exquisitely applied to later steps in secretion and extended to include genetic strategies in the laboratories of James Rothman and Randy Schekman, who were recognized with the Nobel Prize 40 years after George Palade and Albert Claude were similarly honored (Schekman, 2010; Rothman, 2014). The present discussion cannot begin to explore the efforts that have been made to address these and other related questions in the depth that they deserve. In broad terms, however, it is safe to say that one of the primary goals of these efforts has been to extract from the morphologically defined secretory pathway identified by Jamieson and Palade a thorough understanding of the physical components that make it run.

Fortunately for the vitality of modern cell biology, the discovery of the secretory pathway and the elucidation of many of its molecular constituents have been prolific in spawning a vibrant new generation of important and unanswered questions. One of these can be traced directly back to the highly specialized nature of the

experimental system that Jamieson and Palade so elegantly exploited. The pancreatic acinar cell is a factory that is dedicated to producing a number of different digestive enzymes. The vast majority of the protein that it synthesizes is packaged into the zymogen granules that were presciently recognized by Heidenhain as the compartments in which this protein is stored for regulated release (Jamieson and Palade, 1968a). As such, Jamieson and Palade were able to safely assume that essentially all of the radioactive label incorporated into newly synthesized proteins in their pulse chase experiments would follow a common route to a shared destination. Most cells are not similarly single minded. Instead, the secretory pathway must often serve the biosynthetic needs for soluble and membrane proteins of numerous subcellular compartments or extracellular destinations. Thus traffic along the secretory pathway must diverge into multiple streams that carry subsets of the cell's biosynthetic inventory to their particular sites of ultimate functional residence. This in turn implies that the cell is capable of recognizing each of its newly synthesized secretory and membrane proteins and assigning them to the carriers that will mediate their delivery. Furthermore, these sorting processes occur at multiple stages along the secretory pathway.

The requirement for such sorting mechanisms is beautifully epitomized by a scheme that cells employ to direct a subset of newly synthesized lysosomal enzymes to their lysosomes, in which specific recognition and modification of these proteins by a series of enzymes that modify their N-linked sugar chains creates and reveals a mannose-6-phosphate residue (Kornfeld, 1987). This in turn ensures that these newly synthesized lysosomal proteins become substrates for reversible, pH-dependent binding to the mannose-6-phosphate receptor, which allows them to be diverted from the bulk flow of the secretory pathway. Pathological perturbations that prevent the creation of the mannose-6-phosphate signal or its binding to the mannose-6-phosphate receptor causes these newly synthesized lysosomal enzymes to continue to follow the course of the secretory pathway, resulting in their constitutive secretion from the cell. Clearly, such active sorting processes are required to ensure that the secretory pathway not only serves to release secreted proteins from the cell but can also be exploited as a common conveyor that feeds the selective biosynthetic delivery requirements of a variety of cellular destinations.

The need for sorting along the secretory pathway is further illustrated by specialized cell types such as epithelia and neurons. In such polarized cells, the plasma membrane is divided into two or more subdomains, each of which can serve as a site to which selective cohorts of newly synthesized membrane and secretory proteins need to be delivered. An enormous amount has been learned about the pathways and signals that are used to direct biosynthetic protein trafficking in polarized cells. As was first recognized three decades ago, critical steps in the segregation of proteins destined for the apical versus the basolateral surfaces of epithelia or the axonal versus the somatodendritic domains of neurons clearly happens within the *trans*-Golgi network (Griffiths and Simons, 1986). Perhaps not surprisingly, however, the deeper these processes are examined, the more complex they reveal themselves to be (Rodriguez-Boulan *et al.*, 2005; Cao *et al.*, 2012). No single mechanism as simple and elegant as that which accounts for lysosomal enzyme sorting has been revealed to explain polarized sorting. At least in epithelial cells, sorting along the secretory pathway is not limited to the dichotomous allocation of proteins into shared carriers bound for the apical or the basolateral surface. It appears that in many and perhaps in most cases, proteins bound for delivery to the same subdomain of the plasma membrane are transported by distinct cadres

of carrier vesicles that pursue different itineraries and that manifest specific biochemical properties (Farr *et al.*, 2009, 2015; Stoops and Caplan, 2014; Stoops *et al.*, 2015). The growing number and variety of these pathways suggests the intriguing possibility that each newly synthesized protein may follow its own customized variation on the secretory pathway and be packaged into vesicles that carry no other cargoes. The physiological utility of this proliferation of pathways that all connect a common starting point to a shared destination remains to be determined, as do the details of the mechanisms through which this diversity is achieved.

Since its discovery, it has become clear that the secretory pathway performs functions that extend well beyond those of a simple transportation conduit. For example, rigorous quality control mechanisms scrutinize every newly synthesized protein at multiple sites along the secretory pathway, and those proteins found to be misfolded or misassembled are targeted for destruction (Ellgaard and Helenius, 2003). Furthermore, these monitoring mechanisms that are embedded within and along the secretory pathway allow the cell to recognize and adapt to conditions that interfere with proper folding and assembly (Hetz, 2012). A substantial fraction of human genetic diseases is caused by mutations that perturb a particular protein's folding and prevent it from properly traversing the secretory pathway or that pathologically overactivate the signaling and response pathways that cells use to respond to the accumulation of misfolded proteins. The secretory pathway has also adapted itself to the transport of specialized cargoes and the needs of specialized organelles. Recent research, for example, reveals fascinating mechanisms that are deployed to transport very large cargoes, such as polymers of newly synthesized collagen, which could not be physically accommodated within the confines of the carrier vesicles that transport standard cargoes between the secretory pathway's stations (Venditti *et al.*, 2012; Lavieu *et al.*, 2014; Malhotra *et al.*, 2015). Furthermore, at least some proteins, including a subset of those that populate the membrane of the primary cilium, appear to pursue a variant version of the secretory pathway via which they bypass some or all of the structures of the Golgi complex (Hoffmeister *et al.*, 2011; Prydz *et al.*, 2013; Tian *et al.*, 2014). These are but a few of the additional functions and specialized adaptations of the secretory pathway that Jamieson and Palade could not have predicted when they interpreted the tracks of the autoradiographic silver grains that traveled across their electron micrographs half a century ago. On the golden anniversary of their seminal discovery, however, it is perhaps only slightly hyperbolic to suggest that they realized the alchemical feat of turning those silver grains into the gold of a fundamental paradigm of cell biology that promises to generate new questions and fuel new research for at least a half century to come.

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