As cell biologists, like other scientists, we rely on paradigms for guiding our inquiries into how cells function. By paradigm, I don’t mean a specific model, which may be expected in many instances to be overturned altogether, but a deeper conceptual view, interconnected with the technology and scientific language of the times and unquestioned by the majority in the field. For example, the view that all eukaryotic cells have a secretory pathway comprised of a stable endoplasmic reticulum (ER) and Golgi apparatus, using discrete transport vesicles in order to exchange their contents, was long believed to be fundamental for further research. The history of cell biology is marked by acceptance of such paradigms, but also by their gradual evolution.

Having a penchant for philosophy since undergraduate days, I’ve often wondered how paradigms in science come about and what prompts their modification. In my experience, the answer has a lot to do with 1) available technology, which circumscribes what is tested and hypothesized in science, and 2) shared scientific language in a particular field, which influences how findings are related, how scientific phenomena are conceived, and how research questions are framed.

The secretory membrane pathway in my view may be such an example of an evolving paradigm in cell biology. Classic experiments using electron microscopy (Dalton and Felix, 1954; Farquhar et al., 1974; Rambourg et al., 1979) and pulse-chase autoradiographic tracing of newly synthesized proteins (Neutra and Leblond, 1966) set the framework of this paradigm, revealing the progressive movement of newly synthesized secretory proteins from the ER to the Golgi to the cell surface. Small vesicles in the vicinity of the ER and Golgi apparatus seen in transmission electron micrograph cross sections were interpreted as transport intermediates, conveying proteins from one stable station to the next before reaching the plasma membrane. With this framework in hand (Farquhar and Palade, 1981), researchers in the 1980s began addressing the complexity of the intracellular membrane transactions involved in secretory transport, following three distinct research strategies—biochemical, genetic, and imaging approaches.

Pioneering the biochemical approach was James Rothman and colleagues, who used cell-free extracts to reconstitute vesicle formation and fusion of Golgi-derived membranes (Balch et al., 1984). Examining oligosaccharide processing in mixed extracts from mutant “donor” and wild-type “acceptor” Golgi membranes, they found that the virally encoded vesicular stomatitis virus glycoprotein (VSVG) underwent sequential carbohydrate processing, prompting the conclusion that the glycosylation machinery is compartmentalized across
the Golgi’s stack of cisternal elements. In the framework of a stable Golgi system, this led to the idea that vesicles carry VSVG across the Golgi stack in a cis-to-trans direction. Using the in vitro transport assay, Rothman’s group then purified cytosolic components necessary for vesicle budding and membrane fusion (Serafini et al., 1991; Waters et al., 1991; Sollner et al., 1993). Concurrently, Schekman and colleagues spearheaded the genetic approach, isolating conditional lethal, temperature-sensitive secretion mutants and mapping the localization of the corresponding gene products in yeast cells (Novick et al., 1980, 1981). With this, Schekman’s group produced a temporal map of the secretory pathway, identifying the genes and proteins required to operate the secretory pathway (Kaiser and Schekman, 1990). The two approaches soon converged in identifying a core molecular machinery involved in controlling how vesicles form, translocate, and fuse among donor and acceptor compartments of the secretory pathway. The result was a beautiful synergy, reinforcing the prevailing view that protein secretion involves the activity of small transport vesicles for intercompartmental trafficking of proteins. Expressions such as anterograde transport, COPI- and COPII-coated vesicles, small GTPases, and soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) became the indispensable vocabulary in the field.

The third approach—imaging—initially served a supportive role to the dramatic advances made by the biochemical and genetic approaches. Static imaging by electron microscopy (EM) of secretory membranes gave strong support to the idea that COPI vesicles shuttle proteins between stable Golgi cisternae (Orci et al., 1989). However, as the capability to image dynamic processes developed, new ideas emerged. Researchers employing imaging in the late 1980s at first were less interested in the mechanistic basis of secretory vesicle traffic than in the nature of the organelles and vesicle intermediates themselves. Encouraged by the elegant morphology of the Golgi’s elaborate, stack-like structure and the web-like network of the ER, now viewable because of new immunofluorescence techniques, such researchers wanted to address whether organelles are stable, independent structures or more dynamic, relying on other compartments. One hint that the Golgi might not be stable came with the immuno-EM findings by Graham Warren and John Lucocq in the late 1980s showing that the Golgi partially disassembles during mitosis and then reforms (Lucocq and Warren, 1987). However, from the perspective of the governing paradigm, the EM images of scattered mitotic Golgi elements were interpreted as evidence that the Golgi is capable of vesiculating during mitosis and then reforming through the reassociation of these fragments.

Observations with the fungal metabolite brefeldin A (BFA) were more difficult to accommodate. In Rick Klausner’s lab, where I worked as a postdoc, we observed that the Golgi tubulates upon addition of BFA (Lippincott-Schwartz et al., 1990). The tubules carried Golgi proteins into the ER and no apparent Golgi persisted after minutes of BFA treatment. This was the reverse of the forward-only paradigm of vesicle trafficking; moreover, the transport back to the ER did not require conventional coat protein machinery (Donaldson et al., 1990; Orci et al., 1991). When BFA was removed from cells, there emerged a new, fully functional Golgi. These findings did not neatly fit into the biochemical/genetic-based advances in the secretory transport paradigm, in which coated vesicles and small GTPases were seen as central to all trafficking within a stable Golgi system. Instead, the results suggested there are mechanistically distinct anterograde and retrograde trafficking pathways operating between the ER and Golgi (Klausner et al., 1992). These pathways use both vesicular and nonvesicular transport carriers, and a balance in membrane flux between the pathways determined Golgi size and existence.

Some dismissed the BFA results as an artifact of the intervention into cellular processes with the drug. With the advent of the green fluorescent protein (GFP) revolution in which proteins could be tagged in living cells and visualized as they moved through the secretory pathway, it became possible to image the secretory pathway in vivo without BFA. This yielded several surprises in light of the prevailing paradigm. When cargo transport through the secretory pathway was visualized, instead of small vesicles randomly diffusing to and from the different organelles, large tubular-vesicular structures were seen conveying cargo from the ER to Golgi apparatus (Presley et al., 1997). The structures used molecular motors to move along microtubules toward the Golgi and varied in size depending on levels of cargo flux through the pathway. When I first presented movies showing the trafficking of VSVG protein at a conference in 1997, Ben Lewin, then Editor-in-Chief at Cell, memorably asked, “Where are the vesicles?,” to which one member of the audience replied, “They must be invisible with GFP.” The movies revitalized the idea of cisternal progression from EM studies of the 1960s (Morré and Mollenhauer, 2007), since the pleiomorphic transport intermediates appeared to fuse together upon reaching the Golgi apparatus.

However, the GFP-based movies also raised deeper questions. Quantitative measurements of VSVG-GFP trafficking through the secretory pathway by Koty Hirschberg in my lab revealed no change in the rate law for VSVG export out of the ER or Golgi as the number of VSVG molecules in these compartments dropped from >20 million to tens of molecules after temperature release from the ER (Hirschberg et al., 1998). This suggested that the rate-limiting steps in transport do not depend on binary interactions between cargo and specific transport components, which should show saturation effects. One possibility is that they depend instead on lipid phase separation processes, which do not respond to dilution. This fit with emerging membrane lipid research suggesting that the lipid bilayer is not a structurally passive solvent but exhibits lateral segregation potential due to the preferential association among sphingolipids, sterols, and specific proteins (Simons and Ikonnen, 1997). Self-organization of lipids and proteins in the bilayer in this model is believed to induce subcompartmentalization to organize bioactivity of cell membranes. This could drive membrane trafficking events in a way that explains the single rate laws observed for VSVG-GFP trafficking kinetics.

Using GFP-based photobleaching and kinetic modeling approaches, researchers in my lab and Cathy Jackson studied the membrane binding/release kinetics of different coat protein components responsible for cargo sorting into carriers (including COPI, ARF1, ARF-GAP1, and GBF1). All of these proteins underwent fast cytosol/membrane exchange irrespective of vesicle budding (Presley et al., 2002; Liu et al., 2005; Niu et al., 2005). This suggested that the formation of a “coated” carrier occurs on a different time scale than the binding/release cycle of individual coat components, implying that coat lattices are metastable and may not immediately disassemble. This property is similar to that in other filamentous systems, such as microtubules and actin. The effects of metastable coat lattices on membranes are unclear, but one possibility is that they affect protein retention in the Golgi by exerting membrane tension in the bilayer (Antonny, 2006). Biophysical studies of model membranes have shown that membrane tension can drive large-scale phase separation and sorting of lipids and proteins (Baumgart et al., 2003; Roux et al., 2005; Manneville et al., 2008). Without such tension, sorting/segregation of molecules is disrupted. One way to explain the nonselective, directed flow of Golgi membrane components back to the ER under BFA treatment, when coat proteins are dislodged from membranes, is by this type of mechanism.
Using fluorescence recovery after photobleaching to measure the residency time and trafficking pathways of different GFP-tagged Golgi resident components, Theresa Ward in my lab showed that they all were transiently associated with Golgi membranes and underwent either cycling through the ER (i.e., transmembrane enzymes and itinerant membrane components) or rapid exchange with cytosolic pools (i.e., Golgi coat and matrix proteins; Ward et al., 2001). We further demonstrated that within Golgi membranes Golgi enzymes undergo rapid lateral diffusion and are unhindered by extensive interactions that “fix” these proteins within different cisternae (Coe et al., 1996). In addition, Golgi enzymes were shown to move between Golgi elements by membrane tubules (Sciaky et al., 1997), which extended between Golgi subcompartments (Trucco et al., 2004). These findings raised the question of how the Golgi maintains itself as an organelle, retaining its resident components to prevent them from flowing with secretory cargo to the plasma membrane.

Using a fluorescence pulse-chase labeling strategy to quantify cargo export out of the Golgi, Koty Hirschberg and George Patterson in my lab discovered that there is no lag or discrete transit time for cargo transport through the Golgi. Instead, incoming cargo molecules rapidly mixed with those already in the system and exited from partitioned domains at an exponential rate proportional to their total Golgi abundance (resembling radioactive decay; Patterson et al., 2008). This posed a challenge to cisternal progression in its classic form, which predicts that newly arrived cargo exhibits a lag or transit time before exiting the Golgi. Building on the idea that cholesterol-based increases in membrane thickness influence the subcellular distribution of membrane proteins relative to the length of their transmembrane domain (Brecher and Munro, 1993) and that nonrandom lipid architecture is specifically geared to organize functionality within the bilayer (van Meer et al., 2001; Lingwood and Simons, 2010), we constructed a model of intra-Golgi transport to try to account for the new imaging data. In this model, cargo and Golgi-resident enzymes sort spatially due to their preferential affinity for different lipid domains in the Golgi. This, combined with the Golgi’s entry/exit fluxes, stack-like organization, and requirement of vesicular or tubule cargo transport across the stack (which prevents the system from becoming well mixed), results in a dynamic, self-organizing system. Simulation and experimental testing of this rapid partitioning model by Robert Phair and our lab showed that it produces all of the key characteristics of the Golgi apparatus, including polarized lipid and protein gradients, exponential cargo export kinetics, and cargo waves (Patterson et al., 2008).

The basic idea in this partitioning model is that compositional differences within the Golgi are maintained by bidirectional membrane flow. The striking live-cell imaging observations of Golgi dynamics in the yeastSaccharomyces cerevisiae by Ben Glick (Losev et al., 2006) and Aki Nakano (Matsuura-Tokita et al., 2006) showing that isolated Golgi elements undergo maturation fits with this idea (assuming that Golgi elements in yeast [Rambourg et al., 2001] resemble endosomal compartments [Sonnenchsen et al., 2000], which are neither stacked nor have balanced bidirectional flow and so undergo continuous compositional change). Together these data suggest that the Golgi apparatus may not be a conventional organelle in the sense of being an autonomous entity comprised of stable components. Rather, the Golgi seems to function as a steady-state structure undergoing continuous outgrowth from and reconstitution by the ER through bidirectional anterograde and retrograde trafficking (Altan-Bonnet et al., 2004).

In the future, we can expect many new surprises in this field as research uses newer fluorescent probes and imaging techniques to dissect spatial compartmentalization and temporal dynamics of molecules in the secretory pathway. Techniques such as fluorescence resonance energy transfer (FRET) and fluorescence correlation microscopy (FCS) should enable protein–protein interactions in this pathway to be spatially and temporally resolved, revealing potential interactions missed in classical biochemical assays that depend on large, isolatable samples for measurable interactions. Developments in fluorescent probes for better coimaging of proteins (and lipids) and for measuring and perturbing biochemical activities (i.e., GTP hydrolysis) will help in understanding how biochemical activities are organized to drive specific reactions at selected times and places. Finally, advances in superresolution microscopy, such as photoactivated localization microscopy (PALM) (Betzig et al., 2006), stochastic optical reconstruction microscopy (STORM; Rust et al., 2006), stimulated emission depletion microscopy (STED; Hell and Wichmann, 1994), and saturated structured illumination (Gustafsson, 2005), which provide spatial resolutions down to ∼20–60 nm, will enable optical examination of nanometer-scale phenomena of the Golgi and secretory pathway, including vesicle budding and fusion and tubule growth and tethering. These approaches, combined with correlative light-electron microscopy (Polischuk et al., 2000) and biophysical techniques for monitoring membrane curvature, roughness, and tension, are likely to provide important new tools for solving many outstanding questions regarding the overall behavior and function of secretory transport.

From the foregoing, it should be clear that I am convinced that the development of new imaging technology has allowed the field to take a fresh look at the original paradigm of the secretory pathway. It has introduced data described by a vocabulary different from the standard static account of secretory organelles and the vision of discrete transport vesicles. This offers a potentially broader dynamic framework in which to situate the previous biochemical and genetic advances, with room for asking new questions. That, for me, is the excitement of doing science, and I eagerly await the results of research into these questions.

ACKNOWLEDGMENTS
I am grateful to the many dedicated students and postdoctoral fellows and collaborators, including Robert Phair, Cathy Jackson, Mark Terasaki, Carolyn Smith, Eric Siggia, Juan Bonifacino, Eric Betzig, and Harald Hess, with whom I have been privileged to work over the years. I am also grateful to the National Institutes of Health, whose campus has been my scientific home throughout my career.

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