An evolving paradigm for the secretory pathway?

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ABSTRACT The paradigm that the secretory pathway consists of a stable endoplasmic reticulum and Golgi apparatus, using discrete transport vesicles to exchange their contents, gained important support from groundbreaking biochemical and genetic studies during the 1980s. However, the subsequent development of new imaging technologies with green fluorescent protein introduced data on dynamic processes not fully accounted for by the paradigm. As a result, we may be seeing an example of how a paradigm is evolving to account for the results of new technologies and their new ways of describing cellular processes.

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.

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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

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Abbreviations used: BFA, brefeldin A; ER, endoplasmic reticulum; FCS, fluorescence correlation microscopy; FRET, fluorescence resonance energy transfer; GFP, green fluorescent protein; PALM, photoactivated localization microscopy; STED, stimulated emission depletion microscopy; STORM, stochastic optical reconstruction microscopy; VSVG, vesicular stomatitis virus glycoprotein.

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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. Entranced 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 back 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, ArfGAP1, 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 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 (Cole *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 (Bretscher and Munro, 1993) and that nonrandom lipid architecture is specifically geared to organize functionality within the bilayer (van Meer *et al.*, 2008; 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 yeast *Saccharomyces 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 [Sonnichsen *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 reconsumption 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 (Polishchuk *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.

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REFERENCES

Altan-Bonnet N, Sougrat R, Lippincott-Schwartz J (2004). Molecular basis for Golgi biogenesis. Curr Opin Cell Biol 16, 364–372.

Antonny B (2006). Membrane deformation by protein coats. Curr Opin Cell Biol 18, 386–394.

Balch WE, Dunphy WG, Braell WA, Rothman JE (1984). Reconstitution of the transport of protein between successive compartments of the Golgi measured by the coupled incorporation of N-acetylglucosamine. Cell 39, 405–416.

Baumgart T, Hess ST, Webb WW (2003). Imaging coexisting fluid domains in biomembrane models coupling membrane curvature and line tension. Nature 425, 821–824.

Betzig E, Patterson GH, Sougrat R, Lindwasser OW, Olenych S, Bonifacino JS, Davidson MW, Lippincott-Schwartz J, Hess HF (2006). Imaging intracellular fluorescent proteins at near-molecular resolution. Science 313, 1642–1645.

Bretscher MS, Munro S (1993). Cholesterol and the Golgi apparatus. Science 261, 1280–1281.

Cole NB, Smith C, Sciaky N, Terasaki M, Edidin M, Lippincott-Schwartz J (1996). Diffusional mobility of Golgi proteins in membranes of living cells. Science 273, 797–801.

Dalton AJ, Felix MD (1954). Cytologic and cytochemical characteristics of the Golgi substance of epithelial cells of epididymis in situ, in homogenates and after isolation. Am J Anat 94, 171–207.

Donaldson J, Lippincott-Schwartz J, Bloom GS, Kreis TE, Klausner RD (1990). Dissociation of a 110kD peripheral membrane protein from the Golgi apparatus is an early event in brefeldin A action. J Cell Biol 111, 2295–2306.

Farquhar MG, Bergeron JJM, Palade GE (1974). Cytochemistry of Golgi fractions prepared from rat liver. J Cell Biol 60, 8–25.

Farquhar MG, Palade GE (1981). The Golgi apparatus (complex)—(1954–1981)—from artifact to center stage. J Cell Biol 91, 77s–103s.

Gustafsson MG (2005). Nonlinear structured-illumination microscopy: widefield fluorescence imaging with theoretically unlimited resolution. Proc Natl Acad Sci USA 102, 13081–13086.

Hell SW, Wichmann J (1994). Breaking the diffraction resolution limit by stimulated emission: stimulated emission depletion fluorescence microscopy. Opt Lett 19, 780–82.

Hirschberg K, Miller CM, Presley JF, Ellenberg J, Zaal K, Cole NB, Siggia E, Phair, Lippincott-Schwartz (1998). Kinetic and morphological analysis of secretory protein traffic in living cells. J Cell Biol 143, 1485–1503.

Kaiser CA, Schekman R (1990). Distinct sets of *SEC* genes govern transport vesicle formation and fusion early in the secretory pathway. Cell 61, 723–733.

Klausner RD, Donaldson JG, Lippincott-Schwartz J (1992). Brefeldin A: insights into the control of membrane traffic and organelle structure. J Cell Biol 116, 1071–1080.

Matsuura-Tokita K, Takeuchi M, Ichihara A, Mikuriya K, Nakano A (2006). Live imaging of yeast Golgi cisternal maturation. Nature 441, 1007–1010.

Lingwood D, Simons K (2010). Lipid rafts as a membrane-organizing principle. Science 327, 46–50.

Lippincott-Schwartz J, Donaldson J, Schweizer A, Berger E, Hauri HP, Yuan L, Klausner RD (1990). Microtubule-dependent retrograde transport of Golgi proteins into the ER in the presence of brefeldin A suggests an ER recycling pathway. Cell 60, 821–836.

Liu W, Moriyama K, Phair R, Duden R, Lippincott-Schwartz J (2005). In vivo dynamics of ARFGAP1 and its functional interaction with Arf1 and coatomer on Golgi membranes. J Cell Biol 168, 1053–1063.

Losev E, Reinke CA, Jellen J, Strongin DE, Bevis BJ, Glick BS (2006). Golgi maturation visualized in living yeast. Nature 441, 1002–1006.

Lucocq JM, Warren G (1987). Fragmentation and partitioning of the Golgi apparatus in HeLa cells. EMBO J 6, 3239–3246.

Manneville JB, Casella JF, Ambroggio E, Gounon P, Bertherat J, Bassereau P,, Cartaud J, Antonny B, Goud B (2008). COPI coat assembly occurs on liquid-disordered domains and the associated membrane deformations are limited by membrane tension. Proc Natl Acad Sci USA 44, 16946–16951.

Morré DJ, Mollenhauer HH (2007). Microscopic morphology and the origins of the membrane maturation model of Golgi apparatus function. Int Rev Cytol 262, 191–218.

Neutra M, Leblond CP (1966). Radioautographic comparison of uptake of galactose-H3 and glucose-H3 in Golgi region of various cells secreting glycoproteins or mucopolysaccharides. J Cell Biol 30, 137–150.

Niu T-K, Pfeifer AC, Lippincott-Schwartz J, Jackson CL (2005). Dynamics of GBF1, a brefeldin A-sensitive Arf1 exchange factor at the Golgi. Mol Cell Biol 16, 1213–1222.

Novick P, Ferro S, Schekman R (1981). Order of events in the yeast secretory pathway. Cell 25, 461–469.

Novick P, Field C, Schekman R (1980). The identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. Cell 21, 205–215.

Orci L, Malhotra V, Amherdt M, Serafini T, Rothman JE (1989). Dissection of a single round of vesicular transport—sequential intermediates for intercisternal movement in the Golgi stack. Cell 56, 357–368.

Orci L, Tagaya M, Amherdt M, Perrelet A, Donaldson J, Lippincott-Schwartz J, Klausner RD, Rothman J (1991). Brefeldin A, a drug that blocks secretion, prevents the assembly of non-clathrin-coated buds on Golgi cisternae. Cell 64, 1183–1195.

Patterson G, Hirschberg K, Polishchuk R, Gerlich D, Phair RD, Lippincott-Schwartz J (2008). Transport through the Golgi apparatus by rapid partitioning within a two-phase membrane system. Cell 133, 1055–1067.

Polishchuk RS, Polishchuk EV, Marra P, Alberti S, Buccione R, Luini A, Mironov AA (2000). Correlative light-electron microscopy reveals the tubular saccular ultrastructure of carriers operating between Golgi apparatus and plasma membrane. J. Cell Biol 148, 45–58.

Presley JP, Ward T, Miller C, Siggia E, Phair RD, Lippincott-Schwartz J (2002). Dissection of COPI and Arf1 dynamics in vivo and role in Golgi membrane transport. Nature 417, 187–193.

Presley JF, Cole NB, Schroer, Hirschberg, Zaal KJM, Lippincott-Schwartz J (1997). ER to Golgi transport visualized in living cells. Nature 389, 81–85.

Rambourg A, Clermont Y, Hermo L (1979). Three-dimensional architecture of the Golgi apparatus in Sertoli cells of the rat. Am J Anat 154, 455–476.

Rambourg A, Jackson CL, Clermont Y (2001). Three dimensional configuration of the secretory pathway and segregation of secretion granules in the yeast *Saccharomyces cerevisiae*. J Cell Sci 114, 2231–2239.

Roux A, Cuvelier D, Nassoy P, Prost J, Bassereau P, Goud B (2005). Role of curvature and phase transition in lipid sorting and fission of membrane tubules. EMBO J 24, 1537–1545.

Rust MJ, Bates M, Zhuang X (2006). Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). Nat Methods 3, 793–795.

Sciaky N, Presley J, Smith C, Zaal KJM, Cole N, Moreira JE, Terasaki M, Siggia E, Lippincott-Schwartz J (1997). Golgi tubule traffic and the effects of brefeldin A visualized in living cells. J Cell Biol 139, 1137–1156.

Serafini T, Orci L, Amherdt M, Brunner M, Kahn RA, Rothman JE (1991). ADP-ribosylation factor is a subunit of the coat of Golgi-derived COP-coated vesicles: a novel role for GTP-binding protein. Cell 67, 239–253.

Simons K, Ikonen E (1997). Functional rafts in cell membranes. Nature 387, 569–572.

Sollner T, Bennett MK, Whiteheart SW, Scheller RH, Rothman JE (1993). A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. Cell 75, 409–418.

Sonnichsen B, De Renzis S, Nielsen E, Rietdorf J, Zerial M (2000). Distinct membrane domains on endosomes in the recycling pathway visualized by multicolor imaging of Rab4, Rab5 and Rab11. J Cell Biol 149, 901–914.

Trucco A *et al.* (2004). Secretory traffic triggers the formation of tubular continuities across Golgi sub-compartments. Nat Cell Biol 6, 1071–1081.

van Meer G, Voelker DR, Feigenson GW (2008). Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol 9, 112–124.

Ward T, Polishchuk R, Hirschberg K, Barr F, Lippincott-Schwartz J (2001). Maintenance of Golgi structure and function depends on the integrity of ER export. J Cell Biol 155, 557–570.

Waters MG, Serafini T, Rothman JE (1991). Coatomer—a cytosolic protein complex containing subunits of non-clathrin-coated Golgi transport vesicles. Nature 349, 248–251.