

PERSPECTIVES

TIMELINE

The mammalian Golgi — complex debates

Brad J. Marsh and Kathryn E. Howell

Since the first description of the Golgi in 1898, key issues regarding this organelle have remained contentious among cell biologists. Resolving these complex debates, which revolve around Golgi structure–function relationships, is prerequisite to understanding how the Golgi fulfils its role as the central organelle and sorting station of the mammalian secretory pathway.

Although Camillo Golgi described the ‘internal reticular apparatus’ more than 100 years ago¹, many issues regarding the Golgi still remain contentious among cell biologists². In mammalian cells, the Golgi is comprised of a ribbon of flattened stacks of cisternae that are punctuated by openings of various sizes, through which tubules project and vesicles move^{3–5}. Its structure has fascinated cell biologists since the 1950s, when A. J. Dalton and M. D. Felix⁶ first used electron microscopy (EM) to show the basic cisternal arrangement of the organelle. Structural observations of the Golgi have evoked numerous models of how molecules move through this organelle⁷. Additionally, the marker-enzyme hypothesis of C. de Duve⁸ contributed to the development of reliable cell-fractionation techniques and, thereby, to the determination of organelle function. However, the concept that arose from this hypothesis — that a protein ‘resides’ in a specific organelle — slowed our appreciation of how truly dynamic ‘resident’ proteins are.

An enormous amount of data on Golgi structure and function have been gathered over the past 50 years. The challenge now is

to build on previous work — to revise concepts derived from these older data by integrating them with more recent findings that have emerged through the use of sophisticated new technologies. The use of *in-vivo* imaging techniques and green fluorescent protein (GFP)-fusion proteins to study membrane traffic⁹ and of high-resolution, three-dimensional (3D) EM have revealed the dynamic nature, as well as the 3D complexity, of the structure of the Golgi. FIGURE 1 shows the 3D organization of a portion of the Golgi ribbon in a mammalian cell. The general features of the mammalian Golgi are summarized in BOX 1. An improved understanding of the complicated structure of this organelle and of the dynamic nature of molecules that provide its function have led to the development of new concepts, as well as to the re-interpretation of older biochemical, microscopic and cell-free assay data. It has also stimulated a

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re-evaluation of our ideas concerning how the Golgi forms and functions.

The biggest hindrance to progress in understanding the structure–function relationships of the Golgi is that truly fundamental information is missing — not for lack of effort, but because the technology required to obtain the data was not available until relatively recently. This missing information falls into three broad categories — structure, molecular composition (proteins and lipids) and dynamics. More specifically, both the structure and molecular composition must be understood in the context of how they are dynamically modified with function. We believe that resolution of these issues is finally on the horizon. For example, the integration of data from *in-vivo* imaging by light microscopy with high-resolution (~5 nm) 3D structural data derived from EM tomography of rapidly frozen cells/tissue has the potential to resolve structural/dynamic issues. These techniques are continually being improved and promise to yield further insights with the development of better tags that are smaller and less perturbing than GFP and its analogues, and that can be visualized at both the light microscopy and EM levels¹⁰. The application of proteomics techniques, developed relatively recently, will identify the basic protein components of the Golgi and will reveal how these molecules are post-translationally modified^{11,12}. Functional proteomics has the potential to identify many of the molecular changes that occur with changes in physiology or cellular function¹³, and, with the help of informatics scientists, this field promises to move very rapidly. A serious void that remains is our poor understanding of how membrane and signalling lipids contribute to Golgi function, and what kinds of functional modifications they undergo to fulfil these roles.

In 1998, on the 100th anniversary of the discovery of the Golgi, numerous reviews covering the topic of the Golgi were published^{2,14}. These review articles provided detailed

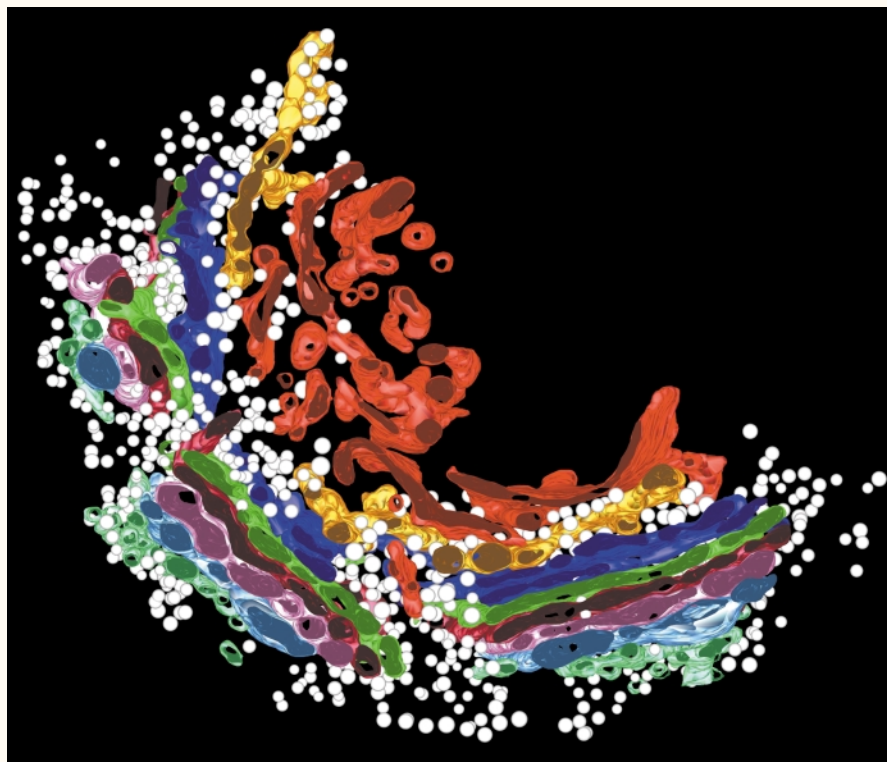


Figure 1 | Model of part of the Golgi ribbon in a mammalian cell generated from a dual-axis three-dimensional reconstruction. Thick (400 nm) sections of plastic-embedded specimens prepared from fast-frozen/freeze-substituted HIT-T15 cells were imaged in an electron microscope (EM) operating at 750 kV. Image data were recorded digitally using semi-automated methods for charge-coupled-device image montage, data acquisition and image alignment as the sample was serially tilted at 1.5° angular increments over a range of 120° ($\pm 60^\circ$) about two orthogonal axes. The three-dimensional (3D) density distributions (tomograms) calculated from each set of aligned tilts were aligned with each other and combined to produce a single 3D reconstruction measuring 5.4 x 4.4 x 0.4 μm^3 . Such reconstructions are readily viewed tomographic slice by tomographic slice. Although each of these computer-generated views resembles a conventional two-dimensional EM image, they correspond to slices much thinner (~4 nm) than can be physically cut. Each Golgi cisterna can be manually segmented, extracted and viewed in any given orientation, and in context with any other modelled object(s), to resolve convoluted membranes. The Golgi ribbon in this model is composed of seven cisternae — *cis*-most (light blue) to *trans*-most (red) — preceded by a layer of docked and fused vesicular tubular clusters (light green). The distribution of the numerous, small non-clathrin-coated and uncoated vesicles (~50 nm, white) is shown in the context of the Golgi cisternae^{5,31}.

accounts of the history associated with work on this curious and complex organelle. Although plant and yeast studies have contributed significantly to our overall understanding of Golgi function, we have limited the scope of this article to the main topics that are now debated in research on the mammalian Golgi. These topics are outlined against the historical backdrop of progress — including recent advances — in the mammalian membrane traffic field. The **TIMELINE** shows a concise historical background to these issues, which are summarized in **BOX 2**.

Golgi formation

As proposed by J. Lippincott-Schwartz and colleagues in 1999 (REFS 15,16), does the mammalian Golgi form by the self-organization of

components as they are exported from the endoplasmic reticulum (ER)? Or, as proposed by G. Warren and colleagues in 2000 (REF. 17), does the Golgi form using a persistent matrix that nucleates Golgi assembly? Historically, the question of organelle biogenesis has been studied primarily in the context of reassembly of the Golgi following mitosis. However, recent data indicate that the time resolution in these earlier studies was too slow to identify the essential features of mitosis, and was certainly too slow to follow formation of the Golgi^{18–21}. The questions of what the Golgi matrix is and how it functions are integral to the topic of Golgi formation and function. These questions have been the subject of significant comment recently, because two well-

respected groups — those of J. Lippincott-Schwartz and G. Warren — have weighed in on opposite sides of this debate^{22–29}. Although their proponents consider the two ideas incompatible, it remains possible that the answer to the question of how the Golgi forms will involve ideas from both sides of the debate, with some Golgi proteins and enzymes redistributing to and from the ER, and other matrix proteins persisting to serve as a ‘seed’ for Golgi reassembly. Resolving these issues will require better definition of the Golgi matrix in both molecular and dynamic terms.

Cargo movement through the Golgi

In the past, this debate has focused on whether anterograde traffic (that is, the forward transport of cargo) through the Golgi is mediated by vesicles, tubules, or the process of cisternal progression/maturation. **FIGURE 2** outlines these mechanisms in the context of the organization of the mammalian Golgi. Experiments to test these mechanisms were originally designed on the basis of the premise that there was a single, exclusive mechanism for anterograde transport. Now, many, but certainly not all, believe that each of these mechanisms (vesicle-, tubule- and cisternal-mediated transport) has an important role in directing traffic forward through the Golgi^{30–32}.

This debate of how cargo is moved through the Golgi is the longest standing, and certainly the most ‘colourful’, in the field of mammalian membrane-traffic research. Between 1958 and the mid-1960s, cisternal progression/maturation was the favoured mechanism for transport through the Golgi^{33–35}. The concept of cisternal progression/maturation was essentially displaced by the vesicular transport model that resulted from the work of J. Jamieson and G. Palade^{36,37}. Their extensive studies, combining pulse-chase analyses, cell fractionation and morphological studies of secretion in the exocrine pancreas, both indicated and favoured the general concept of vesicular transport.

A considerable amount of data that validated the vesicular transport hypothesis were published between the mid-1980s and mid-1990s, predominantly through the collaborative efforts of the groups of J. Rothman and L. Orci³⁸. Many of the details of anterograde vesicular transport were both well established and accepted by the late 1980s. The identification of a component, βCOP , of the coatomer protein complex I (COPI) (a group of seven coat proteins that make up the non-clathrin vesicle coat on Golgi-derived transport vesicles) by R. Duden and

co-workers³⁹ and T. Serafini and colleagues⁴⁰ in 1991 strengthened the vesicular transport model.

The key concepts and identification of regulatory molecules of the COPI-dependent mechanism of vesicular transport came from studies using a cell-free assay to follow the transport of VSV-G (vesicular stomatitis virus membrane glycoprotein). VSV-G is an exogenous transmembrane glycoprotein that has been used extensively to study membrane trafficking through the mammalian secretory pathway. Although retrograde traffic (that is, the transport of molecules backward within the Golgi, and from the Golgi to the ER) was acknowledged, it was only when P. Cosson and F. Letourneur focused on the mechanisms of retrograde transport that it became apparent that one of the functions of COPI vesicles is to mediate the recycling of ER proteins from the Golgi (or from the intermediate compartment between the ER and Golgi) back to the ER^{41,42}. Studies of COPI function and vesicular transport are ongoing, and have resulted in renewed consideration of the validity of the cisternal progression/maturation model.

Over the past few years, considerable evidence has accumulated for non-vesicular mechanisms of transport through the Golgi. Consequently, present research is focused on determining whether these different transport mechanisms act together, or are differentially used and regulated. Does the choice or predominance of a given transport mechanism depend on the cell type, the stage of the cell cycle, the rate of cargo synthesis and the type of cargo synthesized? Although there is

Box 1 | Key features of the mammalian Golgi complex

The key features of the mammalian Golgi complex are that:

- It functions as the central organelle of the cell secretory pathway, and interacts with the endoplasmic reticulum (ER) at both sides of the stack.
- It consists of stacks of flattened cisternae that are connected at equivalent levels across non-compact regions (holes or fenestrae) to form a ribbon.
- Its structure is proposed to be maintained through interactions with a unique matrix.
- It contains enzymes that are involved in the post-translational modification of newly synthesized proteins and lipids (for example, phosphorylation, acylation, glycosylation, methylation and sulphation).
- It contains enzymes that are capable of synthesizing complex sphingolipids and glycolipids.
- Although considered 'resident', Golgi-processing enzymes are in a constant state of dynamic flux between the Golgi, ER, plasma membrane and endosomal compartments.
- At the 'trans' face of the Golgi stack, cargo is sorted for exit to several destinations within the cell and for secretion outside the cell.
- Molecules recycle to the Golgi from the plasma membrane through the endosomal-lysosomal pathway.
- It interacts with all components of the cytoskeleton — microtubules, actin filaments, intermediate filaments, ankyrin and spectrin.
- It forms a 'platform' for many signalling complexes, which, in turn, regulate Golgi function.

considerable evidence that vesicles are a significant component of the transport mechanisms to, within and from the Golgi, it is not yet clear to what extent these other transport mechanisms function as well.

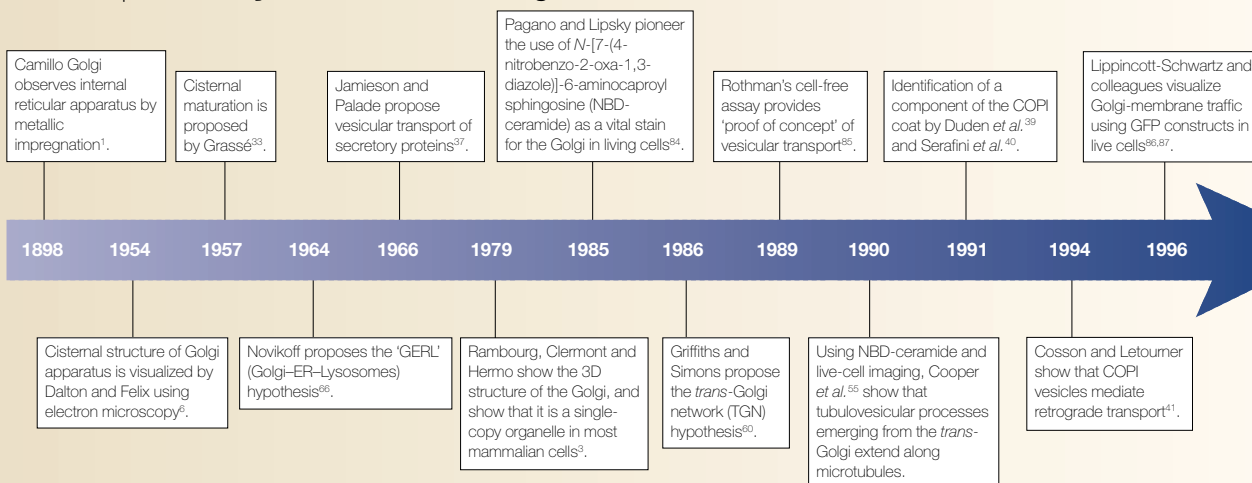
COPI vesicles and bidirectional traffic

The main question now is which sorting and/or trafficking step(s) do COPI vesicles actually mediate? Recent live-cell imaging data using the ϵ subunit of COPI (ϵ COP)—GFP indicate that COPI regulates traffic between the ER and Golgi by generating kinetically stable membrane domains

that give rise to COPI-containing transport intermediates⁴³. Imaging of procollagen-GFP also indicates a role for COPI in regulating cargo export from the ER at a pre-Golgi sorting step⁴⁴. However, as the COPI complex consists of at least seven different proteins, there is every reason to believe that such a complex might have several, related functions — the clathrin paradigm makes this possibility compelling.

Clathrin heavy and light chains function as coat components at the plasma membrane, at the *trans*-Golgi, and, most likely, throughout the entire endocytic pathway.

Timeline | The history of the mammalian Golgi debates



3D, three-dimensional; COP, coatomer protein complex; GFP, green fluorescent protein.

Box 2 | Current debates on Golgi structure–function

The current debates on Golgi structure–function are:

- Is the mammalian Golgi formed *de novo* or by a (persistent) matrix?
- What is the main mechanism of intra-Golgi transport — cisternal progression/maturation, tubules or vesicles?
- Do coatomer protein complex I (COPI) vesicles function in anterograde transport?
- What is the role of tubules in transport to, from and within the Golgi?
- Where and how is cargo sorted for exit from the Golgi to the constitutive, endosomal–lysosomal and regulated secretory pathways?
- What is the definition of the *trans*-Golgi network? Should we re-define it on the basis of current evidence?
- What are the roles of signalling in Golgi function?

However, the function of clathrin-coated vesicles at each of these distinct cellular sites is directed by a plethora of adaptor/effector molecules^{45,46}. The question of whether COPI functions in anterograde intra-Golgi traffic remains controversial. Laboratories involved in this debate present contradictory data using similar assays and methods. J. Rothman and colleagues continue to provide evidence for anterograde-directed vesicle-mediated traffic across the Golgi^{47–50}. However, data from a number of other groups argue against a major role for COPI vesicles in the forward transport of cargo through the mammalian Golgi^{51–53}.

Cargo movement to and from the Golgi

From the 1960s to the mid-1990s, it was well accepted that vesicles mediate traffic to and from the Golgi, and between cisternae. EM micrographs of thin-sectioned cells indicated this, and the vesicular transport model held much appeal. Conceptually, vesicles provide a rational mechanism for sorting, and the routes to and from the Golgi complex are the main areas for sorting in the mammalian secretory pathway. Tubules were, for the most part, not considered, most likely because clear images of tubules were rarely seen in the thin sections used for EM.

As whole-cell immunofluorescence became more widely used — especially when used in combination with perturbants such as **brefeldin A** — a marked variation in our concept of Golgi traffic emerged⁵⁴. Brefeldin A is a fungal metabolite that inhibits the activation of ADP-ribosylation factor 1 (**ARF1**) on Golgi membranes and therefore the assembly of the COPI vesicles, with the result that the transport of newly synthesized proteins from the ER to the Golgi is blocked. *In-vivo* imaging of fluorescent lipids and GFP-hybrid proteins identified both membrane tubules and large, pleiomorphic transport intermediates as major carriers of cargo to

and from the Golgi^{55–59}. Unfortunately, because of the limitations of light microscopy, these studies have not shown to what extent small vesicles also function in these pathways.

The fundamental questions that have arisen are whether tubules and vesicles perform the same functions, and if so, whether they use the same molecular machinery to sort cargo, recruit coats and engage compartmental regulatory systems? Determining the conditions under which each of these transport mechanisms is favoured should provide useful insights into how the function of the Golgi is regulated.

Do tubules also contribute to intra-Golgi transport? Again, this question is difficult to evaluate using *in-vivo* imaging because light microscopy does not provide the resolution that is necessary to distinguish individual elements of the Golgi ribbon and their associated tubules and vesicles. The fluorescence signal within the stack is usually so intense that the Golgi appears as a single, large structure. Furthermore, the frequency with which different laboratories have, so far, observed such tubules by EM indicates that they are unlikely to have a major role in intra-Golgi traffic.

That said, Rambourg and colleagues have shown that dilated tubules/cisternal bridges serve to connect non-equivalent cisternae at points where the Golgi ribbon branches — for example, between a *cis*-cisterna in one region and a *trans*-cisterna in the next region⁷. Such connections could facilitate the movement of molecules forwards and/or backwards through the Golgi to a significant extent. Resolving the question of whether or not tubular/cisternal connections typically exist within the Golgi, and, if so, whether they mediate general or specific transport functions will be imperative to a more complete understanding of how membrane traffic moves through the Golgi.

Sorting and exit from the Golgi

The compartment for sorting cargo for exit from the Golgi was defined as the *trans*-Golgi network (TGN)⁶⁰ by G. Griffiths and K. Simons in 1986. It has also been referred to as the *trans*-Golgi reticulum (TGR)⁶¹. It has been widely accepted by the membrane trafficking community that this compartment is the single *trans*-most cisterna of the Golgi and the network that extends from it. In addition to containing molecules for sorting cargo for exit from the Golgi to the endosomal–lysosomal, regulated secretory and constitutive secretory pathways, the TGN possesses unmistakable structural characteristics. Abundant budding tubular and/or vesicular membrane profiles are coated with clathrin.

Although considerable progress has been made in understanding sorting events at the *trans*-Golgi, the problem is very complex. Most evidence indicates that proteins destined for either the basolateral domain of a polarized cell, or for the endocytic pathway, are sorted through the signal-specific recognition of amino-acid targeting motifs in their cytoplasmic domains⁶². Although molecular studies have provided clues as to the nature of these signals, the complete ensemble of coat proteins and the associated machinery that recognize, sort and form vesicles and tubules in the *trans*-Golgi have not been identified. Even the well-accepted example of clathrin-mediated transport from the *trans*-Golgi to the endosomal–lysosomal pathway has become increasingly complicated as more adaptor complexes and adaptor-like molecules are identified^{25,45,46,63}. Apically directed proteins are proposed to be sorted by their **lectin**-like properties, or by the association of their transmembrane domains with microdomains that are enriched in cholesterol and sphingolipids — referred to as ‘lipid rafts’^{63,64}. Understanding how lipid rafts form and function, and to what extent they contribute to sorting at the *trans*-Golgi, will require the development of ingenious new technologies.

Our own work, using high-resolution 3D EM tomography to study the Golgi complex, has indicated that cargo is sorted not in one but in several *trans*-cisternae that can be reproducibly distinguished from one another by virtue of their structures, coat morphologies and vesicular/tubular profiles⁶⁵. Most recently, this questioning of how the TGN is defined has been supported by data that come from cells prepared using techniques that are better suited to preserving the highly dynamic, yet labile, structure of this organelle⁴⁵. Importantly, these 3D structural

studies of the Golgi have also re-confirmed the close association of specialized ER with *trans*-cisternae. The close apposition of a specialized form of ER with the *trans*-Golgi, first described by A. Novikoff in 1964, was a cornerstone of the structure and function of 'GERL' (Golgi-ER-lysosomes)⁶⁶. The intimate association of this modified ER with the *trans*-Golgi, together with the consistent observation that the *trans*-most Golgi cisterna was positive for **acid phosphatase** (a marker enzyme for lysosomes), led to the formulation of the 'GERL hypothesis'. This hypothesis proposed that lysosomal enzymes bypass the Golgi stack and are delivered directly to the *trans*-most Golgi compartment for the biogenesis of lysosomes. Although the 'GERL' concept 'fell from grace', we believe that elucidating the role(s) of specialized ER in the sorting and exit of cargo from the *trans*-Golgi remains crucial to an overall understanding of Golgi function.

The molecular mechanism(s) for sorting cargo to at least three distinct pathways (apical, basolateral and endosomal-lysosomal) from a single *trans*-Golgi compartment must differ substantially from a process in which cargo destined for each pathway is sorted before entry into multiple, structurally discernable *trans*-cisternae. In fact, it is not yet clear how many vesicle/tubule populations exit the Golgi, and whether this number varies significantly among different cell types. Constitutive trafficking to the apical and basolateral pathways occurs in both polarized and non-polarized cells⁶³, and molecules move to each pathway in different vesicle and tubule populations⁶⁷. Cells that form secretory granules possess an additional regulated secretory pathway, and morphological data indicate that secretory granules might form at different levels within the Golgi stack, and might even exit the Golgi at different sites depending on cell type⁶⁸⁻⁷⁰. In addition, there is now significant evidence that molecules leaving the Golgi move to early, as well as late, endosomes^{71,72}, which would require an additional population of post-Golgi carriers. To confidently address the question of how many different vesicle and tubule populations originate at the Golgi, it will be necessary to track multiple endogenous markers using techniques such as correlative light imaging and 3D EM.

The role of signalling in Golgi function

Our recent understanding of how dynamic the Golgi complex and its many exit pathways are, and of the complexity of its

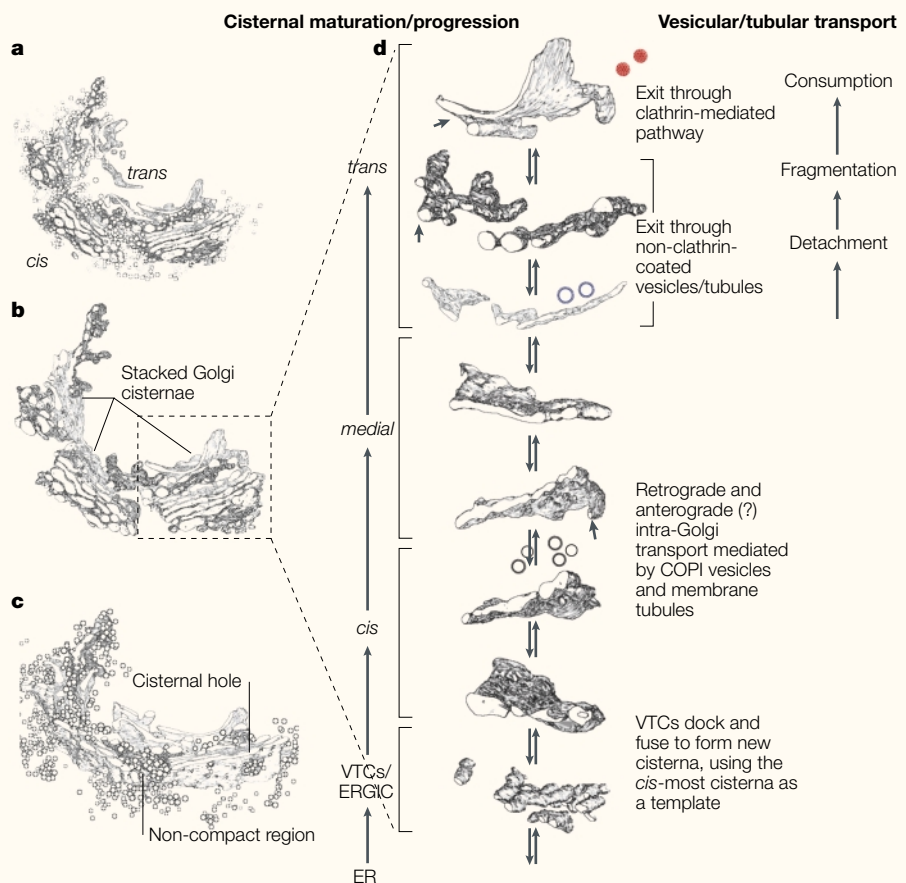


Figure 2 | Dissecting Golgi organization and transport. The structures in **a-d** are from the data presented in FIG. 1. **a** | The orientation of the Golgi ribbon showing the presence of abundant intra-Golgi vesicles. **b** | The orientation of the Golgi ribbon is shown again, but with the vesicles omitted to allow the stacked Golgi cisternae to be visualized more clearly. **c** | These small vesicles (average diameter ~50 nm) fill openings in the Golgi ribbon — wells or non-compact regions — as well as holes within the cisternae themselves, and they are also found at the periphery of stacked Golgi cisternae. The spaces between cisternae are generally too small (≤ 20 nm) to accommodate vesicles. **d** | Cisternae from the boxed region in **b** are shown in the context of the structure-function relationships of the Golgi. In the cisternal maturation/progression model, new cisternae form by the fusion of VTCs (vesicular/tubular clusters)/ERGIC (endoplasmic reticulum-Golgi intermediate compartment) elements that use the existing *cis*-most cisterna as a template. Cisternae progress through the stack and 'mature' as Golgi-processing enzymes are moved backwards to earlier cisternae through a COPI-regulated, vesicle-mediated mechanism. At the *trans*-face of the Golgi, several *trans*-cisternae, frequently referred to as the *trans*-Golgi network — depleted of early resident Golgi enzymes — detach and fragment as membrane is consumed in the process of packaging cargo for exit. Exit is mediated by vesicles and/or tubules. COP, coatomer protein complex; ER, endoplasmic reticulum.

interactions with many molecules of the cytoskeletal network, has made us aware that these events must be coordinately regulated by an incredibly sophisticated system. Many regulatory molecules localized to the Golgi have been shown to affect its function. The role of ARF1 is the best studied, and there is compelling data to support a function for ARF1 in the assembly, sorting and disassembly of COPI vesicles^{73,74}. The functions of ARF1 are, in turn, regulated by large families of guanine nucleotide exchange factors (GEFs) and guanine nucleotide-activating proteins

(GAPs)⁷⁵. ADP-ribosylation factors also modify the signalling lipids within the Golgi membrane bilayer, and might additionally function to coordinate interactions with the cytoskeleton^{76,77}.

Members of other signalling protein families that are found associated with the Golgi have been shown to modulate its function. These include the Rab protein family (in particular, **Rab1** and **Rab2** at the *cis*-Golgi, **Rab6** throughout the stack, and **Rab9**, **Rab11** and **Rab17** at the *trans*-Golgi⁷⁸), the heterotrimeric G proteins⁷⁹, phosphatidylinoside 3-kinases and **CDC42** (REF. 80). Protein

kinase D (PKD) has been shown to be associated with the Golgi, and quite recently it was reported that PKD is recruited to the Golgi by diacylglycerol⁸¹. This recruitment was shown to be involved in the formation of transport intermediates between the Golgi and plasma membrane⁸¹. To integrate much of this information, it has been proposed that the Golgi actually functions as a signalling platform that regulates membrane traffic, cytoskeletal organization⁸² and signal transduction⁷⁵. However, this big picture is lacking evidence and support, and global approaches, such as functional proteomics combined with informatics, will be essential to provide and integrate the information on signalling.

Conclusion and perspectives

Many issues discussed in this article are being resolved through the application of new preparative and imaging techniques — developed at both the light microscopy and EM level — and the integration of this information with biochemical data obtained using new, exquisitely sensitive analytical techniques. It is clear that no technique is without its limitations. The principle on which the field of cell biology was founded — that integrating data from multiple techniques and approaches is necessary to establish concepts — still holds.

Given the significant differences in Golgi structure that are observed among different cell types⁷, we must seriously question whether anybody still expects that a single ‘universal’ transport model will prevail. A better understanding of Golgi function might be achieved by distinguishing common functional features of those Golgi structures that vary with cell type. In the foreseeable future, the Golgi research community will no longer rely on the expression of exogenous proteins such as VSV-G to study membrane trafficking and sorting. Overexpression experiments will become completely out of date as endogenous proteins are biosynthetically or post-translationally tagged with very small molecules that can not only be followed by light microscopy, but that can also be visualized at the EM level¹⁰.

Certainly more challenging, but also on the horizon, is the prospect of being able to detect and identify macromolecular complexes by virtue of their structure alone, in the context of high-resolution 3D cellular data⁸³. We also anticipate that better methodologies will be developed to study the contributions of lipids to Golgi-membrane traffic, and that informatics systems

will effectively integrate all the data obtained from genomics and functional proteomics. A global understanding of how traffic is regulated through the Golgi, in the context of all of the regulatory events throughout the cell, will take some time to achieve, and this, almost certainly, will be our greatest challenge.

Brad J. Marsh is at the Boulder Laboratory for 3D EM, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309, USA.

Kathryn E. Howell is at the Department of Cellular and Structural Biology, University of Colorado School of Medicine, Denver, Colorado 80262, USA.

Correspondence to K.E.H.
e-mail: Kathryn.Howell@UCHSC.edu

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- Golgi, C. Sur la structure des cellules nerveuses des ganglions spinaux. *Arch. Ital. Biologie* **30**, 278–286 (1898).
- Farquhar, M. G. & Palade, G. E. The Golgi apparatus: 100 years of progress and controversy. *Trends Cell Biol.* **8**, 2–10 (1998).
- Rambourg, A., Clermont, Y. & Hermo, L. Three-dimensional architecture of the Golgi apparatus in Sertoli cells of the rat. *Am. J. Anat.* **154**, 455–476 (1979).
- Ladinsky, M. S., Mastronarde, D. N., McIntosh, J. R., Howell, K. E. & Staehelin, L. A. Golgi structure in three dimensions: functional insights from the normal rat kidney cell. *J. Cell Biol.* **144**, 1135–1149 (1999).
- Marsh, B. J., Mastronarde, D. N., Buttle, K. F., Howell, K. E. & McIntosh, J. R. Organellar relationships in the Golgi region of the pancreatic β cell line, HIT-T15, visualized by high resolution electron tomography. *Proc. Natl Acad. Sci. USA* **98**, 2399–2406 (2001).
- Dalton, A. J. & Felix, M. D. Cytological and cytochemical characteristics of the Golgi substance of epithelial cells of the epididymis *in situ*, in homogenates and after isolation. *Am. J. Anat.* **94**, 171–208 (1954).
- Rambourg, A. & Clermont, Y. in *The Golgi apparatus*. (eds Berger, E. G. & Roth, J.) 37–61 (Birkhauser Verlag, Basel, 1997).
- de Duve, C. The role of lysosomes in the pathogeny of disease. *Scand. J. Rheumatol. Suppl.* 63–66 (1975).
- Lippincott-Schwartz, J., Roberts, T. H. & Hirschberg, K. Secretory protein trafficking and organelle dynamics in living cells. *Annu. Rev. Cell Dev. Biol.* **16**, 557–589 (2000).
- Gaietta, G. *et al.* Multicolor and electron microscopic imaging of connexin trafficking. *Science* **296**, 503–507 (2002).
- Taylor, R. S. *et al.* Proteomics of rat liver Golgi complex: minor proteins are identified through sequential fractionation. *Electrophoresis* **21**, 3441–3459 (2000).
- Bell, A. W. *et al.* Proteomics characterization of abundant Golgi membrane proteins. *J. Biol. Chem.* **276**, 5152–5165 (2001).
- Wu, C. C. *et al.* GMx33: a novel family of trans-Golgi proteins identified by proteomics. *Traffic* **1**, 963–975 (2000).
- Berger, E. G. & Roth, J. *The Golgi apparatus* (Birkhauser Verlag, Basel, Switzerland, 1997).
- Zaal, K. J. *et al.* Golgi membranes are absorbed into and reemerge from the ER during mitosis. *Cell* **99**, 589–601 (1999).
- Ward, T. H., Polishchuk, R. S., Caplan, S., Hirschberg, K. & Lippincott-Schwartz, J. Maintenance of Golgi structure and function depends on the integrity of ER export. *J. Cell Biol.* **155**, 557–570 (2001).
- Seemann, J., Jokitalo, E., Pypaert, M. & Warren, G. Matrix proteins can generate the higher order architecture of the Golgi apparatus. *Nature* **407**, 1022–1026 (2000).
- Cole, N. B., Sciaky, N., Marotta, A., Song, J. & Lippincott-Schwartz, J. Golgi dispersal during microtubule disruption: regeneration of Golgi stacks at peripheral endoplasmic reticulum exit sites. *Mol. Biol. Cell* **7**, 631–650 (1996).
- Lucocq, J. M., Pryde, J. G., Berger, E. G. & Warren, G. A mitotic form of the Golgi apparatus in HeLa cells. *J. Cell Biol.* **104**, 865–874 (1987).
- Ho, W. C., Allan, V. J., van Meer, G., Berger, E. G. & Kreis, T. E. Reclustering of scattered Golgi elements occurs along microtubules. *Eur. J. Cell Biol.* **48**, 250–263 (1989).
- Miles, S., McManus, H., Forsten, K. E. & Storie, B. Evidence that the entire Golgi apparatus cycles in interphase HeLa cells: sensitivity of Golgi matrix proteins to an ER exit block. *J. Cell Biol.* **155**, 543–555 (2001).
- Wells, W. A. Let's make Golgi. *J. Cell Biol.* **155**, 498–499 (2001).
- Misteli, T. The concept of self-organization in cellular architecture. *J. Cell Biol.* **155**, 181–185 (2001).
- Lowe, M. Golgi Complex: biogenesis *de novo*? *Curr. Biol.* **12**, R166–R167 (2002).
- Pfeffer, S. R. Constructing a Golgi complex. *J. Cell Biol.* **155**, 873–875 (2001).
- Check, E. Cell biology: will the real Golgi please stand up. *Nature* **416**, 780–781 (2002).
- Glick, B. S. Can the Golgi form *de novo*? *Nature Rev. Mol. Cell Biol.* **3**, 615–619 (2002).
- Barr, F. A. The Golgi apparatus: going round in circles? *Trends Cell Biol.* **12**, 101–104 (2002).
- Glick, B. S. ER export: more than one way out. *Curr. Biol.* **11**, R361–R363 (2001).
- Mironov, A. A., Weidman, P. & Lugini, A. Variations on the intracellular transport theme: maturing cisternae and trafficking tubules. *J. Cell Biol.* **138**, 481–484 (1997).
- Marsh, B. J., Mastronarde, D. N., McIntosh, J. R. & Howell, K. E. Structural evidence for multiple transport mechanisms through the Golgi in the pancreatic β -cell line, HIT-T15. *Biochem. Soc. Trans.* **29**, 461–467 (2001).
- Pelham, H. R. & Rothman, J. E. The debate about transport in the Golgi — two sides of the same coin? *Cell* **102**, 713–739 (2000).
- Grassé, P. P. Ultrastructure polarite reproduction de l'appareil de Golgi. *C. R. Acad. Sci.* **245**, 1278–1281 (1957).
- Mollenhauer, H. H. & Whaley, W. G. An observation on the functioning of the Golgi apparatus. *J. Cell Biol.* **17**, 222–225 (1963).
- Mollenhauer, H. H. & Morre, D. J. The tubular network of the Golgi apparatus. *Histochem. Cell Biol.* **109**, 533–543 (1998).
- Palade, G. Intracellular aspects of the process of protein secretion. *Science* **189**, 347–358 (1975).
- Jamieson, J. D. & Palade, G. E. Role of the Golgi complex in the intracellular transport of secretory proteins. *Proc. Natl Acad. Sci. USA* **55**, 424–431 (1966).
- Rothman, J. E. Mechanisms of intracellular protein transport. *Nature* **372**, 55–63 (1994).
- Duden, R., Griffiths, G., Frank, R., Argos, P. & Kreis, T. E. β -COP, a 110 Kd protein associated with non-clathrin-coated vesicles and the Golgi complex, shows homology to β -adaplin. *Cell* **64**, 649–665 (1991).
- Serafini, T. *et al.* A coat subunit of Golgi-derived non-clathrin-coated vesicles with homology to the clathrin-coated vesicle coat protein beta-adaplin. *Nature* **349**, 215–220 (1991).
- Cosson, P. & Letourneur, F. Coatamer interaction with di-lysine endoplasmic reticulum retention motifs. *Science* **263**, 1629–1631 (1994).
- Letourneur, F. *et al.* Coatamer is essential for retrieval of dilysine-tagged proteins to the endoplasmic reticulum. *Cell* **79**, 1199–1207 (1994).
- Presley, J. F. *et al.* Dissection of COPI and Arf1 dynamics *in vivo* and role in Golgi membrane transport. *Nature* **417**, 187–193 (2002).
- Stephens, D. J. & Pepperkok, R. Imaging of procollagen transport reveals COPI-dependent cargo sorting during ER-to-Golgi transport in mammalian cells. *J. Cell Sci.* **115**, 1149–1160 (2002).
- Robinson, M. S. & Bonifacino, J. S. Adaptor-related proteins. *Curr. Opin. Cell Biol.* **13**, 444–453 (2001).
- Boehm, M. & Bonifacino, J. S. Genetic analyses of adaptin function from yeast to mammals. *Gene* **286**, 175–186 (2002).
- Orci, L., Amherdt, M., Ravazzola, M., Perrelet, A. & Rothman, J. E. Exclusion of Golgi residents from transport vesicles budding from Golgi cisternae in intact cells. *J. Cell Biol.* **150**, 1263–1270 (2000).
- Orci, L. *et al.* Anterograde flow of cargo across the Golgi stack potentially mediated via bidirectional ‘percolating’ COPI vesicles. *Proc. Natl Acad. Sci. USA* **97**, 10400–10405 (2000).
- Volchuk, A. *et al.* Megavesicles implicated in the rapid transport of intracisternal aggregates across the Golgi stack. *Cell* **102**, 335–348 (2000).
- Orci, L. *et al.* Bidirectional transport by distinct populations of COPI-coated vesicles. *Cell* **90**, 335–349 (1997).
- Martinez-Menarguez, J. A. *et al.* Peri-Golgi vesicles

- contain retrograde but not anterograde proteins consistent with the cisternal progression model of intra-Golgi transport. *J. Cell Biol.* **155**, 1213–1224 (2001).
52. Mironov, A. A. *et al.* Small cargo proteins and large aggregates can traverse the Golgi by a common mechanism without leaving the lumen of cisternae. *J. Cell Biol.* **155**, 1225–1238 (2001).
 53. Lanoix, J. *et al.* Sorting of Golgi resident proteins into different subpopulations of COPI vesicles: a role for ArfGAP1. *J. Cell Biol.* **155**, 1199–1212 (2001).
 54. Klausner, R. D., Donaldson, J. G. & Lippincott-Schwartz, J. Brefeldin A: insights into the control of membrane traffic and organelle structure. *J. Cell Biol.* **116**, 1071–1080 (1992).
 55. Cooper, M. S., Cornell-Bell, A. H., Chernjavsky, A., Dani, J. W. & Smith, S. J. Tubulovesicular processes emerge from *trans*-Golgi cisternae, extend along microtubules, and interlink adjacent *trans*-Golgi elements into a reticulum. *Cell* **61**, 135–145 (1990).
 56. Polishchuk, R. S. *et al.* Correlative light-electron microscopy reveals the tubular-sacculus ultrastructure of carriers operating between Golgi apparatus and plasma membrane. *J. Cell Biol.* **148**, 45–58 (2000).
 57. Hirschberg, K. *et al.* Kinetic analysis of secretory protein traffic and characterization of Golgi to plasma membrane transport intermediates in living cells. *J. Cell Biol.* **143**, 1485–1503 (1998).
 58. Toomre, D., Keller, P., White, J., Olivo, J. C. & Simons, K. Dual-color visualization of *trans*-Golgi network to plasma membrane traffic along microtubules in living cells. *J. Cell Sci.* **112**, 21–33 (1999).
 59. Presley, J. F. *et al.* ER-to-Golgi transport visualized in living cells. *Nature* **389**, 81–85 (1997).
 60. Griffiths, G. & Simons, K. The *trans* Golgi network: sorting at the exit site of the Golgi complex. *Science* **234**, 438–443 (1986).
 61. Geuze, H. J., Slot, J. W., Strous, G. J., Hasilik, A. & von Figura, K. Possible pathways for lysosomal enzyme delivery. *J. Cell Biol.* **101**, 2253–2262 (1985).
 62. Matter, K., Yamamoto, E. M. & Mellman, I. Structural requirements and sequence motifs for polarized sorting and endocytosis of LDL and Fc receptors in MDCK cells. *J. Cell Biol.* **126**, 991–1004 (1994).
 63. Keller, P. & Simons, K. Post-Golgi biosynthetic trafficking. *J. Cell Sci.* **110**, 3001–3009 (1997).
 64. Simons, K. & Ikonen, E. Functional rafts in cell membranes. *Nature* **387**, 569–572 (1997).
 65. Ladinsky, M. S., Kremer, J. R., Furciniti, P. S., McIntosh, J. R. & Howell, K. E. HVEM tomography of the *trans*-Golgi network: structural insights and identification of a lace-like vesicle coat. *J. Cell Biol.* **127**, 29–38 (1994).
 66. Novikoff, A. B. GERL, its form and function in neurons of rat spinal ganglia. *Biol. Bull.* **127**, 358 (1964).
 67. Bennett, M. K. *et al.* Perforated cells for studying intracellular membrane transport. *Methods Cell Biol.* **31**, 103–126 (1989).
 68. Bainton, D. F. & Farquhar, M. G. Origin of granules in polymorphonuclear leukocytes. Two types derived from opposite faces of the Golgi complex in developing granulocytes. *J. Cell Biol.* **28**, 277–301 (1966).
 69. Slot, J. W. *et al.* Glucose transporter (GLUT-4) is targeted to secretory granules in rat atrial cardiomyocytes. *J. Cell Biol.* **137**, 1243–1254 (1997).
 70. Orci, L. *et al.* Direct identification of prohormone conversion site in insulin-secreting cells. *Cell* **42**, 671–681 (1985).
 71. Futter, C. E. *et al.* In polarized MDCK cells basolateral vesicles arise from clathrin- γ -adaptin-coated domains on endosomal tubules. *J. Cell Biol.* **141**, 611–623 (1998).
 72. Xiang, Y., Molloy, S. S., Thomas, L. & Thomas, G. The PC6B cytoplasmic domain contains two acidic clusters that direct sorting to distinct *trans*-Golgi network/endosomal compartments. *Mol. Biol. Cell* **11**, 1257–1273 (2000).
 73. Eugster, A., Frigerio, G., Dale, M. & Duden, R. COP I domains required for coatomer integrity, and novel interactions with ARF and ARF-GAP. *EMBO J.* **19**, 3905–3917 (2000).
 74. Lanoix, J. *et al.* GTP hydrolysis by arf-1 mediates sorting and concentration of Golgi resident enzymes into functional COP I vesicles. *EMBO J.* **18**, 4935–4948 (1999).
 75. Donaldson, J. G. & Lippincott-Schwartz, J. Sorting and signaling at the Golgi complex. *Cell* **101**, 693–696 (2000).
 76. De Matteis, M. A. & Morrow, J. S. The role of ankyrin and spectrin in membrane transport and domain formation. *Curr. Opin. Cell Biol.* **10**, 542–549 (1998).
 77. Godi, A. *et al.* ARF mediates recruitment of PtdIns-4-OH kinase- β and stimulates synthesis of PtdIns(4,5)P₂ on the Golgi complex. *Nature Cell Biol.* **1**, 280–287 (1999).
 78. Martinez, O. & Goud, B. Rab proteins. *Biochim. Biophys. Acta* **1404**, 101–112 (1998).
 79. Denker, S. P., McCaffery, J. M., Palade, G. E., Insel, P. A. & Farquhar, M. G. Differential distribution of α subunits and $\beta\gamma$ subunits of heterotrimeric G proteins on Golgi membranes of the exocrine pancreas. *J. Cell Biol.* **133**, 1027–1040 (1996).
 80. Wu, W. J., Erickson, J. W., Lin, R. & Cerione, R. A. The γ -subunit of the coatomer complex binds Cdc42 to mediate transformation. *Nature* **405**, 800–804 (2000).
 81. Baron, C. L. & Malhotra, V. Role of diacylglycerol in PKD recruitment to the TGN and protein transport to the plasma membrane. *Science* **295**, 325–328 (2002).
 82. Chabin-Brion, K. *et al.* The Golgi complex is a microtubule-organizing organelle. *Mol. Biol. Cell* **12**, 2047–2060 (2001).
 83. Bohm, J. *et al.* Toward detecting and identifying macromolecules in a cellular context: template matching applied to electron tomograms. *Proc. Natl Acad. Sci. USA* **97**, 14245–14250 (2000).
 84. Lipsky, N. G. & Pagano, R. E. A vital stain for the Golgi apparatus. *Science* **228**, 745–747 (1985).
 85. Malhotra, V., Serafini, T., Orci, L., Shepherd, J. C. & Rothman, J. E. Purification of a novel class of coated vesicles mediating biosynthetic protein transport through the Golgi stack. *Cell* **58**, 329–336 (1989).
 86. Cole, N. B. *et al.* Diffusional mobility of Golgi proteins in membranes of living cells. *Science* **273**, 797–801 (1996).
 87. Sciaky, N. *et al.* Golgi tubule traffic and the effects of brefeldin A visualized in living cells. *J. Cell Biol.* **139**, 1137–1155 (1997).

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 Online links

DATABASES

The following terms in this article are linked online to:

Swiss-Prot: <http://www.expasy.ch/>
acid phosphatase | ARF1 | brefeldin A | CDC42 | eCOP | GFP | lectin | PKD | Rab 1 | Rab2 | Rab6 | Rab9 | Rab11 | Rab17

FURTHER INFORMATION

Kathryn E. Howell's lab:

<http://www.uchsc.edu/gsc/cdb/faculty/KathrynH/Index.html>

The Boulder laboratory for 3D fine structure:

<http://bio3d.colorado.edu/>

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TIMELINE

Birth of the life sciences in Spain and Portugal

Neidhard Paweletz

For many centuries, Spain and Portugal were occupied by various nations. The consequent mixing of cultures formed a unique environment in which to do science.

The Iberian Peninsula was, for many centuries, a welcome target for foreign powers. Romans and Visigoths ruled it from AD ~200–700 and oppressed the Iberians. The most influential, however, were the Arabs and, in AD 710, the Arabian army that had marched across North Africa reached the Strait of Gibraltar. In AD 711, the Arab commander Tarik and his army passed the Strait and defeated the Visigoths at Guadalete. From here, the Islamic invasion continued and expanded across large parts of the Iberian Peninsula.

The army was soon followed by Arabian scholars, physicians, artists and other educated people, who established new centres of Islamic culture. Córdoba became the capital of the Islamic empire *al-Andalus* (Andalusia), and other important centres of science and scholarship were established; among these, Toledo became especially important¹ (BOX 1). Christian beliefs of the time proclaimed that sickness occurred according to God's will and that humans were not allowed to interfere with God's action. So, sick people were neither

diagnosed nor treated; rather, monks or nuns nursed them until they got better or died. Although there were some medical activities by non-clerics (such as barbers) and monasteries often had a herbal garden in which plants for medical use were grown, the progress of medicine in Western Europe had ground to a halt. Arabian scholars, by contrast, were not influenced by Christian beliefs and so they began to establish new centres of wisdom and practical skills². Arabian medicine blossomed and produced outstanding physicians and surgeons who became well known in the Western world.

Here, I recall some of the discoveries that emerged and describe some of the scholars who worked in Spain and Portugal between the sixth and twentieth centuries (see TIMELINE).

Isidore of Seville

In the sixth and seventh centuries, Seville was a focus of 'compilation' — the conservation of ancient knowledge. Isidore of Seville was born around AD 560/570 (REFS 3,4). He learned Greek in a monasterial or episcopal school in Seville and, in AD 599, became Bishop of Seville and Catholic Primate of Spain. During this time, he wrote many books on various aspects of human life. Particularly important are his