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Antti Rivinoja, Francois M. Pujol, Antti Hassinen & Sakari Kellokumpu

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REVIEW ARTICLE

## Golgi pH, its regulation and roles in human disease

ANTTI RIVINOJA, FRANCOIS M. PUJOL, ANTTI HASSINEN & SAKARI KELLOKUMPU

Department of Biochemistry, University of Oulu, Oulu, Finland

### Abstract

Most organelles within the exocytic and endocytic pathways typically acidify their interiors, a phenomenon that is known to be crucial for their optimal functioning in eukaryotic cells. This review highlights recent advances in our understanding of how Golgi acidity is maintained and regulated, and how its misregulation contributes to organelle dysfunction and disease. Both its biosynthetic products (glycans) and protein-sorting events are highly sensitive to changes in Golgi luminal pH and are affected in certain human disease states such as cancers and cutis laxa. Other potential disease states that are caused by, or are associated with, Golgi pH misregulation will also be discussed.

**Key words:** Disease, glycosylation, Golgi, pH regulation

### Introduction

Eukaryotic cells have evolved multiple systems to compartmentalize their functions into membrane-bound organelles. This allows them to complete numerous reactions simultaneously with high precision and speed. A key issue in this functional optimization is the ability to generate an optimal microenvironment for each process. For example, while mitochondria are alkaline and use this pH gradient to synthesize ATP, organelles within the secretory and endocytic pathways typically are acidic (1–3). Organellar acidity is used to regulate and maintain many fundamental cellular processes that range from vesicular trafficking to post-translational processing and protein sorting, import of nutrients, pathogens, and toxins, as well as degradation in lysosomes (4–13). The pH dependency of these processes has traditionally been studied using organellar pH gradient-dissipating drugs, such as monensin (a  $\text{Na}^+/\text{H}^+$  ionophore), chloroquine (a weak base), and bafilomycin A (a vacuolar H-ATPase inhibitor).

Organelle acidity is in general thought to be regulated by three main ion transport systems. These are ATP-mediated proton pumping, counter-ion

conductance, and intrinsic proton leakage (2,3,14–19). Protons ( $\text{H}^+$ ) are pumped into membranous compartments by the vacuolar  $\text{H}^+$  ATPase (V-ATPase). Counter-ion conductance is used to prevent the build-up of excess positive charge (membrane potential) via passive influx of anions (e.g. chloride) and/or efflux of another cation (e.g. potassium). Intrinsic  $\text{H}^+$  leakage across organellar membranes in turn counteracts proton import and prevents excessive over-acidification of the organelle lumen (15). Therefore, it may contribute significantly to the observed variations in the steady-state pH of the organelles within the secretory and endocytic pathways (Figure 1). A pertinent question that specifically concerns the Golgi apparatus is how the pH is uniquely regulated along the cis-trans axis of the organelle. Golgi pH regulation also poses another problem. This relates to its functioning as a central station between the secretory and endocytic pathways, receiving and sending vesicular carriers between these two functionally different but converging membrane systems. How the Golgi apparatus can deal with such challenges, and maintain its slightly acidic physiological pH, will be discussed next. Later we will discuss how its misregulation contributes to human disease.

**Key messages**

- Golgi pH is strictly regulated.
- Misregulation of Golgi pH induces glycosylation defects.
- Golgi pH is misregulated in cancer cells and cutis laxa.

**The Golgi apparatus and the secretory pathway**

An Italian scientist, Camillo Golgi, initially described the Golgi apparatus in 1898 as an odd reticular structure in nerve cells (20). The Golgi apparatus is now recognized as a crucial component of the

secretory pathway (Figure 1) that also converges with the endocytotic pathway (21,22). Together, these two pathways are responsible for the various biosynthetic (secretory pathway) and degradative processes (endocytotic pathway). They form a dynamic, constantly fluctuating, polymorphic intracellular membrane system (23–25), so that the exact borders of each organelle are sometimes difficult to define.

According to the current view, however, the Golgi apparatus begins from or after the endoplasmic reticulum–Golgi intermediate compartment (ERGIC) (26,27) and can be divided into three main compartments: the cis-Golgi network (CGN), the Golgi stack of cisternae (cis-Golgi, medial-Golgi, trans-Golgi), and the trans-Golgi network (TGN). Under the electron microscope the Golgi cisternae look like flattened pleiomorphic discs with dilated rims and

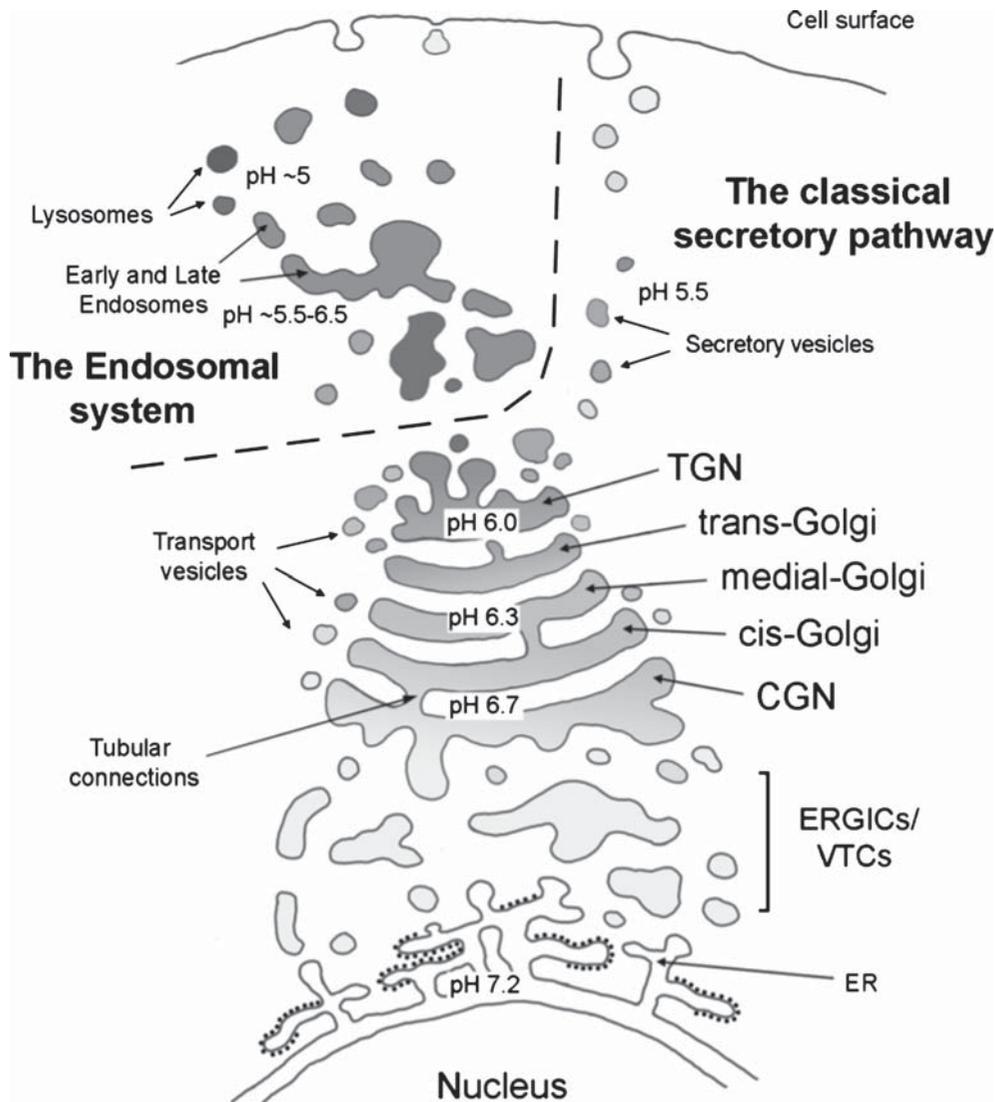


Figure 1. Schematic representation of the compartmentalized secretory (biosynthetic) and endocytic (degradative) pathways and their convergence at the level of the Golgi apparatus (modified from (146)). Note the different steady-state pH values of each organelle. ERGIC is a pleiomorphic membrane structure between the ER and the Golgi, where proteins are arrested at 15°C (26).

adjacent vesicles. Tubular extrusions sometimes connect neighboring cisternae and even adjacent stacks (28–33). The CGN and the TGN, in turn, consist of tubular networks, whose lateral regions are occupied by budding and fusing vesicles (21,22,34). A typical higher eukaryotic cell contains multiple Golgi stacks that assemble into a peri- or juxtannuclear structure around the microtubule-organizing center (MTOC) (35,36). Each Golgi stack of cisternae is an independent unit where several post-synthetic modifications take place, such as glycosylation and sulfation of proteins and lipids (37–40). It also has a role in (glyco) lipid biosynthesis (41), in protein and lipid sorting, as well as in cell polarization (23,25,41,42).

Due to the dynamic nature and continuous trafficking of newly synthesized and recycling proteins and lipids, the maintenance of the Golgi architecture and functions requires constant remodeling. Although the mechanisms are not fully understood, it is known that cytoskeletal proteins and Golgi-specific matrix proteins are involved and appear to provide a protein scaffold for the cisternal assembly and architecture. Remodeling also requires the presence and correct functioning of many luminal and transmembrane proteins as well as strict control of vesicular trafficking and luminal pH. Once all these factors are under control, the Golgi apparatus can perform its many functions with astonishing precision and speed.

### Golgi acidification by the vacuolar H<sup>+</sup> ATPase

Golgi pH measurements with several different fluorescence-based approaches (2,3,14–19) have shown that the Golgi luminal pH is mildly acidic and decreases along the cis-trans axis of the Golgi stack, from pH 6.7 in the CGN (entry face) to pH 6.0 in the TGN (exit face). As in other acidic organelles, the maintenance of the Golgi luminal pH in each of these Golgi subcompartments is thought to reflect changes in the rates between proton pumping, counterion conductance, and proton leakage across the organelle membranes. While all these main ion transport systems appear to be common to acidic organelles, additional components or various protein isoforms may be required in each case. In the next paragraphs, proteins that are currently known to regulate Golgi pH are described.

Golgi acidity is generated by the vacuolar H<sup>+</sup> ATPase (V-ATPase), an ATP-powered proton pump (43–45). This has been convincingly demonstrated by using V-ATPase-specific inhibitors such as bafilomycin A and concanamycin A. Treatment of cells with these drugs results in complete neutralization of the Golgi lumen within 10–20 min. Both in yeast and mammalian cells, the V-ATPase is a multisubunit protein complex that consists of a peripheral

domain V<sub>1</sub> and an integral membrane domain V<sub>0</sub> (Figure 2). The cytosolic V<sub>1</sub> domain is composed of eight subunits (with an A<sub>3</sub>B<sub>3</sub>CDE<sub>2</sub>FG<sub>2</sub>H<sub>1-2</sub> stoichiometry), whereas the integral V<sub>0</sub> domain has six subunits (with an a<sub>1</sub>c<sub>4</sub>c'<sub>1</sub>c''<sub>1</sub> stoichiometry) (43). Some of these subunits may differ between V-ATPases that localize in different cellular compartments. For example, the subunit a isoform Stv1p is found in Golgi-localized V-ATPases in yeast, while the Vph1p isoform is found in V-ATPases that localize to vacuoles (43). These data suggest that different organelles may possess structurally and perhaps also functionally different V-ATPase variants.

All the V-ATPases, however, are structurally and functionally similar to the mitochondrial F<sub>0</sub>F<sub>1</sub> ATP synthase (F-ATPase), but unlike F-ATPase, which synthesizes ATP in a proton gradient-dependent manner, V-ATPase utilizes ATP to transport protons against its concentration gradient, i.e. into the organelle lumen. Hydrolysis of ATP within the V<sub>1</sub> domain induces a rotatory movement of a c-ring in the integral V<sub>0</sub> domain, which transports two protons, one at a time, from the cytosolic hemi-channel to a luminal hemi-channel formed by the a subunit of the V<sub>0</sub> domain (for further details, see Jefferies et al. (43) and references therein).

In the presence of excess ATP, the V-ATPase is able to acidify the Golgi lumen well below its steady-state pH (e.g. our unpublished data). This

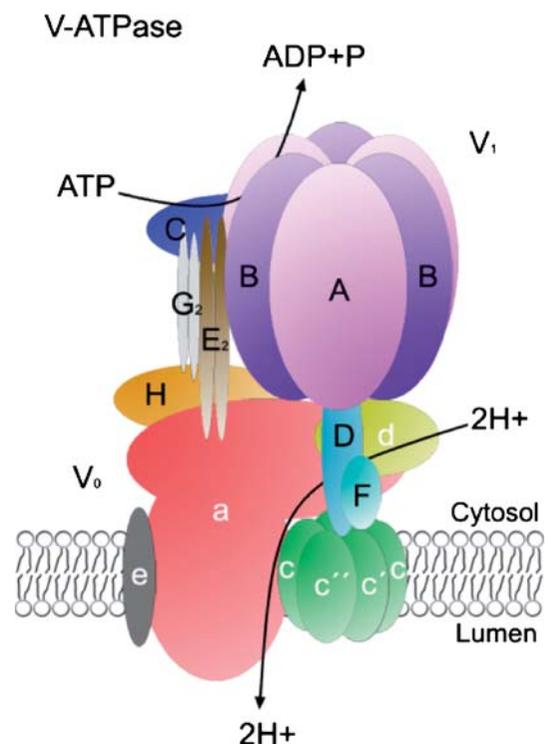


Figure 2. The multisubunit structure of the V-ATPase (modified from (146)). For further details, see text.

observation suggests that the density of the V-ATPase in Golgi membranes is not a limiting factor for Golgi acidity. In addition to density, the V-ATPase activity can also be regulated by other means, such as glucose-mediated reversible assembly and disassembly of the  $V_1$  and  $V_0$  domains, re-shuffling of disulfide bonds within the ATP binding A subunit, changes in coupling between ATP hydrolysis and  $H^+$  pumping (43), or by regulatory proteins, such as the V-ATPase accessory subunit Ac45(46). However, because the physiological role of the V-ATPase activity regulation is unclear in most cases (besides energy consumption), and the fact that V-ATPase can over-acidify the Golgi lumen, it cannot be considered as the primary factor that determines the Golgi pH (1–3,15,17).

### Counter-ion conductance

In all acidic organelles, the primary role of counter-ion conductance, i.e. influx of anions or efflux of cations, is to maintain the organelle membrane potential low despite continuous  $H^+$  import by the V-ATPase (2,47,48). In its absence, membrane potential would increase (inside positive), which in turn would lead to inhibition of V-ATPase activity and to limited organelle acidity (47). Therefore, low counter-ion conductance can modulate the extent of organelle acidification. This is the case in endosomes (48–50), in which defects in the chloride channel protein 5 (CIC-5) are associated with defective endosomal acidification in the kidney (51). In contrast, counter-ion conductivity of the Golgi membranes is considered to be high enough to support proton pumping (1–3,15,17). Therefore, counter-ion conductance in the Golgi appears not to be a limiting factor for acidification. Rather, it serves as an indispensable ‘background component’ that, by preventing an increase in membrane potential, enables normal  $H^+$  pumping and Golgi acidification to or below its steady-state pH.

The transport protein that most likely mediates counter-ion conductance in the Golgi was recently reported to be an anion channel protein, called the Golgi pH regulator (GPHR)(52). It was discovered by using an elegant screen of mutants with reduced secretion and altered glycosylation of cell surface constituents. Mutation of the GPHR was shown to result in delayed transport of newly synthesized proteins from the Golgi to the plasma membrane, impaired glycosylation of proteins, and structural disorganization of the Golgi apparatus. Direct measurement of the steady-state Golgi pH revealed that the mutation of GPHR decreased acidification in the Golgi by about 0.4–0.5 pH units without affecting lysosomal pH, endocytosis, or recycling. Physiological

studies with recombinant GPHR showed that it may be identical to the two previously characterized Golgi-localized anion channels, GOLAC-1 and GOLAC-2 (53,54). All three channels have five to six conductance states that are mostly open, allowing efficient counter-ion transport. They are also weakly anion-selective ( $P_{Cl^-}/P_{K^+}$  permeability ratio  $2.8 \pm 1.3$ ), voltage and DIDS (4,4'-Diisothiocyanato-2,2'-stilbenedisulfonic acid)-sensitive, and pH-insensitive. However, no data were presented to demonstrate that the GPHR alters chloride permeability of the Golgi membranes or that the lack of acidification is due to the absence of GPHR-mediated counter-ion conductance. Thus, the possibility that GPHR regulates Golgi pH by some other means cannot be excluded.

Many other ion transporters have been localized in the Golgi. These include the cystic fibrosis transmembrane conductance regulator (CFTR) (55,56), a mid-1-related chloride channel (MCIC) (57), and voltage-gated chloride channels (CICs) (51,58–60). CFTR is a cAMP-regulated chloride channel which localizes to the trans-Golgi, the TGN, endosomes, and prelysosomes (55). A mutation in the CFTR gene has been associated with defective acidification of these organelles (55,56) and will be discussed in more detail later. MCIC is an anion channel (or an activator of anion channels) that localizes to the endoplasmic reticulum (ER), the Golgi, and the nucleus (57). However, its role in Golgi pH regulation awaits confirmation. CICs, on the other hand, comprise a large family of voltage-gated chloride channels, which are often required for proper acidification of intracellular organelles (51,59). Thus far, two CICs, CIC-3B in mammalian cells (60) and Gef-1 in yeasts(58), have been detected in the Golgi membranes, but their roles in Golgi pH regulation remain unclear. Golgi membranes are also highly permeable to  $K^+$ (3).  $K^+$  conductance may be at least partly GPHR-mediated (52,53), but other  $K^+$  conductive channels may also coexist in the Golgi. An apparent role for such a  $K^+$  channel would be to expel  $K^+$  and serve as an alternative counter-ion conductance pathway during active proton pumping by the V-ATPase. Golgi membranes also contain the  $Na^+/K^+$ -ATPase (61). Even though no clear function has been assigned to it yet, it may contribute to Golgi membrane potential via unbalanced  $K^+/Na^+$  transport and thereby limit Golgi acidification by the V-ATPase. Finally, it should also be noted that the concentrations of anions or cations are interdependent and can be influenced by the transport of  $Ca^{2+}$ ,  $Mg^{2+}$ , or  $Mn^{2+}$ , all of which are present in the Golgi lumen at high concentrations. The latter two cations serve also as important co-factors for several Golgi glycosyltransferases (62).

### Golgi buffering: AE2 $\text{HCO}_3^-/\text{Cl}^-$ exchanger

In addition to the proteins described above, other factors such as cargo transit through the Golgi, vesicular transport, and luminal buffering may also affect Golgi pH (2). Since the Golgi is located in the center of the secretory pathway, it is constantly exposed to the transit of multiple transporters and channel proteins destined for endosomes, lysosomes, and the plasma membrane. Some of the transiting proteins are fully active soon after their biosynthesis in the ER (2,63) and may thus affect Golgi pH. In this respect, vesicular trafficking is also a challenge, as the Golgi resides in the cross-roads between the secretory and endocytic pathways. The vesicles from the endosomal compartments bring acidic loads, whereas those from the ER bring alkaline loads to the Golgi. For the above reasons, the Golgi lumen must be able to adapt rapidly to changing pH conditions.

To confront such pH-related challenges, cells have evolved various buffering systems. One such system is thought to consist of luminal matrix proteins that are rich in chemical groups with suitable  $\text{pK}_a$  values, i.e. groups that protonate and deprotonate around physiological Golgi pH. Soluble buffering molecules, such as  $\text{HCO}_3^-$ , are continuously transported via specific transporters across the plasma membrane to balance cytoplasmic alkaline or acid loads and may thus also be used in the maintenance of Golgi pH (1). This idea is supported by the identification of an erythrocyte anion exchanger 1 (band3, AE1, SLC4A1) homolog in the Golgi membranes of several cell types (64,65). More recent work has identified this transporter as the AE2a isoform (SLC4A2a) (66,67) of the AE gene family, members of which are all known as electroneutral  $\text{HCO}_3^-/\text{Cl}^-$  exchangers. They normally localize in the plasma membrane where they regulate cytosolic pH, chloride concentration, and cell volume (68,69). Our most recent data (Rivinoja et al., unpublished) also provide direct evidence to show that the AE2a exchanger is indeed involved in Golgi pH regulation. AE2a over-expression in COS-7 cells was found to raise Golgi pH by about 0.4 pH units. Bicarbonate also slowed ATP-mediated Golgi acidification by the V-ATPase. Thus, by importing base ( $\text{HCO}_3^-$ ) equivalents, the AE2a can increase Golgi buffering capacity and prevent Golgi over-acidification. Because the exchanger resides almost exclusively in the Golgi membranes (64), it seems to represent a unique system that is utilized only by this organelle.

### Intrinsic proton leakage

The fourth component that is involved in Golgi pH regulation is the rate at which protons exit this compartment. As cellular membranes contain components

that render them intrinsically permeable to protons,  $\text{H}^+$  exit pathways play an important role in balancing  $\text{H}^+$  pumping. Previous studies by Wu et al. (15) have suggested that the decrease in pH between successive secretory compartments is likely due to decreased proton efflux and may be coupled with an increased density of the V-ATPase. Although the 'channel' protein itself remains to be identified, proton efflux may involve either exchange of luminal protons for cytosolic cations, free proton conductivity, or elimination of protons by base import (1,2). Physiological measurements have shown that the characteristics of this putative  $\text{H}^+$  channel are reminiscent of those of the plasma membrane-localized  $\text{H}^+$  channels. Proton export from the TGN was also found to be voltage-sensitive and inhibited by  $\text{Zn}^{2+}$ , suggesting the involvement of a regulated channel (3,70). However,  $\text{Zn}^{2+}$  inhibition was only partial. Thus, the presence of residual  $\text{Zn}^{2+}$  resistance may indicate the existence of another, yet unknown, proton leakage pathway. Two ubiquitously expressed and Golgi-localized  $\text{Na}^+/\text{H}^+$  exchanger (NHE) isoforms, NHE7 and NHE8 (71,72), may also function in proton export. The  $\text{Na}^+/\text{H}^+$  exchange is driven by ion gradients and may occur when  $\text{H}^+$  concentration in the Golgi lumen is high enough to drive the influx of cytosolic  $\text{Na}^+$ . In support of this, over-expression of both NHE7 and NHE8 were found to increase  $\text{Na}^+$  and  $\text{K}^+$  influx to the Golgi (71). Both also significantly raised luminal Golgi pH (72). These data are consistent with an NHE-mediated 'proton leakage' pathway that involves exchange of luminal protons for cytosolic sodium or potassium ions. The existence of this pathway, however, awaits further confirmation, since NHE8 was recently reported to regulate the morphology and function of late endosomes instead of the Golgi (73).

The suggested AE2-mediated import of  $\text{HCO}_3^-$  (in exchange for  $\text{Cl}^-$  efflux) into the Golgi lumen may also provide an intriguing means to expel protons from the Golgi lumen. It is well known that  $\text{HCO}_3^-$ , in the presence of protons (as in the Golgi lumen), is readily converted via a short-lived carbonic acid intermediate into  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . These end-products can rapidly cross lipid membranes via diffusion ( $\text{CO}_2$ ) or aquaporin channels ( $\text{H}_2\text{O}$ ), thereby expelling protons. At pH 6.3, half of the  $\text{HCO}_3^-$  is estimated to be in the form of  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . This represents a sizeable  $\text{H}^+$  leak and could provide a channel-free proton exit pathway across the Golgi membranes without any extra energy cost.

### An integrated model for Golgi pH regulation

An integrated but intentionally simplified model of how Golgi pH is regulated by the various ion transport

systems is presented in Figure 3. In the presence of ATP, protons are continuously pumped into the Golgi lumen by the V-ATPase. This is facilitated by a simultaneous counter-anion ( $\text{Cl}^-$ ) influx (or alternatively via cation efflux), most likely via the GPHR ion channel. Once the Golgi pH becomes sufficiently acidic ( $\text{pH} < 6.3$ ), proton efflux balances  $\text{H}^+$  pumping and prevents further acidification of the Golgi lumen. Proton efflux can be mediated by an as yet unidentified proton 'channel' and/or by AE2a-mediated  $\text{HCO}_3^-$  import. In the latter case, luminal  $\text{HCO}_3^-$  and  $\text{H}^+$  then can form membrane-permeable  $\text{H}_2\text{O}$  and  $\text{CO}_2$  via a spontaneous and reversible chemical reaction to eliminate protons. The suggested  $\text{HCO}_3^-$ -based model offers several benefits for the maintenance of the steady-state pH in the Golgi and, thus, also for its normal functioning. It represents an electroneutral and protein-free proton efflux pathway across the Golgi membranes to balance  $\text{H}^+$  pumping and to prevent over-acidification of the Golgi lumen. Its end-products,  $\text{H}_2\text{O}$  and  $\text{CO}_2$ , can also be reutilized in the cytoplasm by converting them back to  $\text{H}^+$  and  $\text{HCO}_3^-$  via the activity of carbonic anhydrase II. Moreover, due to the high permeability of both end-products, the system will rarely become saturated. Future work will be needed to unravel the true nature of the  $\text{H}^+$  leakage 'channel' in the Golgi membranes and in other acidic organelles as well.

### Golgi pH misregulation and diseases

Correct glycosylation, i.e. the addition of sugar structures to proteins and lipids in the ER and the Golgi apparatus, is known to be critical for a variety of basic cellular functions and biological recognition events, ranging from the regulation of gene expression to protein folding, cell signaling, immunological

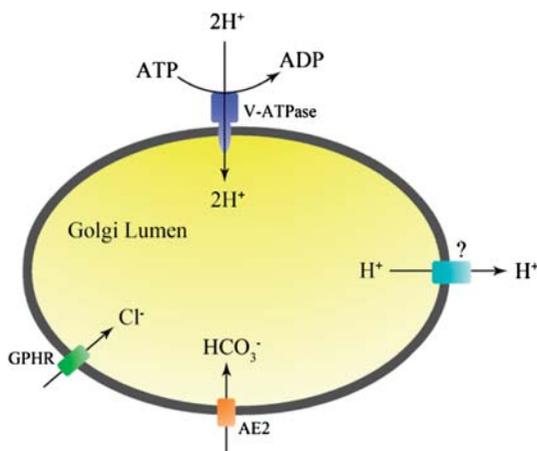


Figure 3. An integrated view of Golgi pH regulation with the main ion transport systems involved.

defense, cell motility, and the development of multicellular organisms (74–77). While much progress has been made in unraveling how the highly compartmentalized, yet dynamic Golgi structure and vesicular trafficking are maintained in a mammalian cell, we still poorly understand how this organelle can faithfully construct a multitude of different sugar structures (glycans) that are often cell-, tissue-, organ-, or organism-specific; yet, if defective for some reason, a disease may develop. This is highlighted by the identification of several glycosylation-associated disease states, such as congenital diseases of glycosylation (CDGs), inflammation, cancers, and diabetes (78–83). In most cases, defective glycosylation has been traced back to mutations or altered expression of Golgi glycosylation enzymes (i.e. glycosyltransferases, glycosidases) or to nucleotide sugar transporters required to provide substrates for glycan synthesis. In other cases, however, the underlying molecular defects are less apparent, and the challenge now is to identify these, which may lead to future medical interventions. One such defect is the misregulated Golgi pH, which has been shown to be directly associated in only two disease states (cancers and cutis laxa). In other cases, direct evidence is missing and warrants further investigation.

### Cancer

Altered glycosylation is a hall-mark of most cancers (84–88). These changes include the expression of truncated and hypersialylated O-linked oligosaccharide chains (89–92), reduced sulfation (93), reduced O-acetylation of sialic acid (94,95), elevated fucosylation (96), altered branching of N-glycans (97,98), and increased expression of blood group antigens (99). Some of these glycosylation alterations, such as increased expression of truncated sialyl-Tn (Neu5Ac $\alpha$ 2-6GalNAc $\alpha$ 1-Ser/Thr) or T-antigens (core 1 Gal $\beta$ 1-3GalNAc $\alpha$ 1-Ser/Thr) (92,100–102), are associated with increased proliferation, invasion, and metastasis of many cancer cell types and are therefore useful prognostic markers for malignancy. Most likely, by modulating interactions of cells with other cells or the extracellular matrix, altered glycosylation can also modulate overall cell behavior. Consistent with this view, terminal Gal $\beta$ 1-3/4GalNAc and/or sialic acid may facilitate interactions with galectins (soluble proteins that recognize N-acetyl-lactosamine-like (Gal $\beta$ 1-3/4GalNAc $\alpha$ 1-Ser/Thr) structures (144)) and siglecs (type I membrane proteins that bind to sialic acid containing glycans (145)) that have established roles in cellular recognition and signaling events and are also known to modulate e.g. innate and adaptive immune responses (87,103).

In several cases, cancer-associated glycosylation alterations arise because of changes in the expression of the corresponding glycosyltransferases (38,104–106). Often, however, such strict correlations are lacking (102,107–111). Other causes therefore must exist and may involve e.g. increased expression of nucleotide sugar transporters, such as the UDP-Gal transporter in cases where T-antigen expression is increased (112). In other cases, the absence of glycosyltransferase-specific chaperones, such as the core 1  $\beta$ 1,3-galactosyl transferase-specific molecular chaperone Cosmc, has been associated with increased Tn-antigen expression, albeit in a non-cancerous specimen (113). Changes in the splicing of the acceptor glycoprotein CD44 has also been shown to be associated with T-antigen expression both in cancerous and normal tissues (114).

Cancer-associated glycosylation changes may also result from more general defects in Golgi function and pH homeostasis. The first observations that led to this idea came from studies in which treatment of cells with pH gradient dissipating drugs were found to increase the expression of the cancer-associated T-antigen (6,8,10,12,85), most likely due to a lack of further glycosylation of this core 1 O-glycan structure. Golgi architecture was also changed, showing a fragmented morphology (12). Thereafter, direct Golgi pH measurements with a fluorescent marker (GT-EGFP) protein (19) in breast and colorectal cancer cells (MCF-7, HT-29, SW-48) confirmed that the Golgi pH is slightly more alkaline ( $-0.2$ – $0.4$  pH units) than in non-malignant cells (115). Moreover, the observed Golgi pH increase was found to correlate well with increased T-antigen expression in the cells. The observation (115) that only a 0.2 pH unit increase, i.e. less than that detected in cancer cells, is sufficient to impair terminal glycosylation of both N- and O-glycans provides further support for the involvement of an altered Golgi pH in cancer-associated glycosylation abnormalities. Moreover, we have recently found that, besides T-antigen expression, other glycosylation changes typical for cancer cells are induced by raising Golgi pH only slightly (Heiskanen et al., unpublished). However, not all glycosylation events are sensitive to Golgi pH changes. For example,  $\alpha$ 2,6-sialylation (in contrast to  $\alpha$ 2,3-sialylation) is not affected by a slight Golgi pH increase. Because these two sialylation events are competitive, this observation may explain why carcinoembryonic antigen (CEA) extracted from colon cancer tissue carries  $\alpha$ 2,6-linked sialic acid (97,116) and not  $\alpha$ 2,3-linked sialic acid as in normal cells.

How does an altered Golgi pH then impair glycosylation? We have recently shown that, in contrast to our expectations, this is not due to a

pH-dependent inactivation of Golgi glycosyltransferases, which in general have acidic pH optima, nor to the limited availability of the sugar nucleotides (117). Rather, it was found that the slight Golgi pH changes induced by 20–40  $\mu$ M chloroquine resulted in mislocalization of certain terminal glycosyltransferases, which accumulate in late endosomes due to their higher than normal pH (118). Similar observations with other Golgi proteins and enzymes have also been demonstrated using more potent drugs such as  $\text{NH}_4\text{Cl}$  and bafilomycin A (10,119). These observations suggest that the acidic Golgi pH is primarily required to localize correctly glycosyltransferases and glycosidases in the different Golgi subcompartments. However, this holds true only for certain enzymes, as not all are sensitive to moderate changes in Golgi pH. We anticipate that other clues such as the observed oligomerization (120) may explain the differential pH sensitivity of the enzymes.

Besides glycosylation, abnormal Golgi pH in cancer cells likely contributes also to other phenotypic changes seen in cancer cells. For example, Golgi pH changes have been shown to interfere with the apical sorting of proteins in epithelial cells (7) and thus may provide a link to loss of cell polarity in cancer cells. Moreover, the extent of the acidification of the Golgi and endosomal compartments in breast cancer cells has been associated with the development of multidrug resistance (121–123). Many of these chemotherapeutic drugs are weak bases and become protonated and sequestered in acidic organelles in non-malignant and multidrug-resistant cells, while in drug-sensitive cells this does not happen, because the organelles are less acidic. As a result, the drugs can bind to DNA and other cellular structures in drug-sensitive cells and eventually cause cell death. These observations emphasize how a better understanding of organelle pH regulation may help develop new strategies to combat multidrug-resistance and cancers.

### Cutis laxa

Autosomal recessive cutis laxa type II is a congenital disease that is also related to Golgi pH misregulation. The disease involves excessive wrinkling of the skin at an early age and is caused by mutations in the  $\alpha$ 2 subunit of V-ATPase coded by the gene ATP6V0A2 (124). Patients suffering from the related wrinkly skin syndrome carry the same mutations but without the symptoms of elastin deficiency (125). The mutations result in a complete loss of V-ATPase function, thereby preventing Golgi acidification. Definitive evidence as to what extent the mutation impairs Golgi acidification, however, remains unclear,

as Golgi pH was not directly measured in the study, and the Golgi also seems to co-express another isoform of the  $\alpha$  subunit (124). The mutations were also shown to impair retrograde trafficking of Golgi vesicles to the ER, most likely by inhibiting their fusion with the ER membrane. As expected, the processing of terminal glycans in the trans-Golgi was impaired. This was evidenced by the general lack of sialic acids in complex type N-glycans as well as in O-glycans in the affected individuals (124,126,127). The terminal glycosylation defects or the general Golgi dysfunction have been linked to structural changes in ECM proteins, such as fibulin 5 and elastin, which are responsible for the elasticity of the skin (125). In cutis laxa patients, defective N- and O-glycosylation also leads to a delayed development of the fetal central nervous system. Although the exact causes for the cutis laxa phenotype have remained unclear, it presents a new type of inherited disorder (CDG-II) that is almost certainly caused by misregulation of Golgi pH in the affected cells. One complicating factor is the fact that the  $\alpha 2$  subunit has also been shown to localize in early endosomes in cells from cutis laxa patients (128), which indicates that altered endosomal pH and functioning may contribute to disease etiology as well.

### Cystic fibrosis

Cystic fibrosis (CF) is the most common lethal inherited disease in Caucasians. Hall-marks of CF include viscous mucus in respiratory epithelia, bacterial colonization, pancreatic dysfunction, high levels of sweat chloride, and infertility in males. The mutation that causes the CF has been traced to a cAMP-regulated chloride channel, termed the cystic fibrosis transmembrane conductance regulator (CFTR). Normally, this apical membrane channel protein allows transmembrane flow of anions, and therefore also water, into and out of cells. In CF, the CFTR-mediated anion flow is drastically reduced, resulting in the production of more viscous mucus. Altered glycosylation of the mucus is also considered to play an important role in disease etiology (129,130). A good indication of this is the fact that the main oligosaccharide-binding protein of *Pseudomonas aeruginosa* (a pathogen that typically colonizes lungs in CF) preferentially binds to altered glycans found in CF patients. These glycans are typically hyperfucosylated, hypersulfated, and hyposialylated, when compared with control samples (80). However, discrepant findings, such as reduced sulfation, increased sialylation, and reduced fucosylation of CF mucins, have also been reported (131). Therefore, it is currently not clear to which glycan structures the lung-colonizing bacteria predominantly bind.

An interesting link between the mutated CFTR, the observed glycosylation changes, and Golgi acidification was originally proposed by Barasch et al. (55,132) who reported that in CF cells the TGN was slightly more alkaline (by 0.2 pH units) than the TGN of normal counterparts. According to this scenario, mutations in the CFTR chloride channel likely impair counter-anion conductance, leading to defective acidification of the TGN and altered terminal glycosylation as observed in CF patients. However, this 'defective Golgi acidification hypothesis' soon turned out to be controversial, as other research groups have found no evidence that the CFTR actually affects Golgi pH (130,133–136). Even more confusing was the observation that the pH of the TGN in CF cells is hyperacidified relative to control cells. By using fluorescence ratio imaging, the trans-Golgi pH in several cell lines was found to be 0.2–0.5 pH units lower in CF cells than in healthy or genetically matched rescued cells (61,137). Moreover, hyperacidification of the TGN and endosomal compartments was found to be  $\text{Na}^+$ -dependent (61), suggesting that CFTR may modulate organelle pH indirectly via other ion transporters or channels. Thus, by which mechanism, or if at all, CFTR may regulate Golgi pH remains to be clarified. The main challenge in CF, therefore, is to unravel what other roles CFTR may be playing in intracellular membranes and identify the underlying causes for altered glycosylation in CF, if not an altered Golgi pH. One possibility, as suggested by Schulz et al. (131), may be accelerated degradation of CF mucin in the affected patients. How accelerated degradation would result in the increased terminal sialylation of CF mucins that was observed in the same study remains unclear.

### Hailey–Hailey disease

Congenital mutations of secretory pathway  $\text{Ca}^{2+}$ -ATPases (SPCAs) cause skin disorders that are characterized by blisters and erosions in flexural areas of the body. One of these diseases, called the Hailey–Hailey disease, is caused by a mutation in one allele of the human SPCA1 gene, ATP2C1 (138,139). SPCA1 has been shown to transport both  $\text{Ca}^{2+}$  and  $\text{Mn}^{2+}$  into the Golgi lumen and therefore plays an important role in the cytosolic and intra-Golgi cation homeostasis (140). The decreased concentration of these cations in the Golgi lumen in affected cells is thought to lead to impaired glycosylation, proteolytic processing, folding, trafficking, or sorting of proteins that are essential for cell-to-cell adhesion (141,142). These defects may explain why the affected patients are unable to maintain structurally intact desmosomes and epidermis. The fact that

Mn<sup>2+</sup> is an important co-factor for many glycosyltransferases residing in the Golgi (143) supports this assumption. Because Mn<sup>2+</sup> at high concentrations is also cytotoxic, and SPCA1 is the only known Mn<sup>2+</sup> transporter, it also plays an important role in sequestering excess Mn<sup>2+</sup> from the cytoplasm to the extracellular space. In addition, the luminal concentrations of both Ca<sup>2+</sup> and Mn<sup>2+</sup> are likely to be dependent on the concentration of other ions or may themselves be involved in counter-ion conductance, raising the possibility that Golgi pH may also be affected. Glycan analyses and direct Golgi pH measurements are required to clarify their involvement in the disease etiology.

### Concluding remarks

Maintenance of a mildly acidic pH in the Golgi has turned out to be crucial for its normal functioning and, in particular, for its glycosylation potential. Therefore, any defect in glycosylation, in principle, can result from Golgi pH misregulation. Although complex, Golgi pH regulation seems to involve mainly four ion transport systems. These include V-ATPase, GPHR, AE2a, and the still poorly characterized proton leakage 'channel'. Of these, GPHR and AE2-mediated Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup> transport, respectively, appear to be specific for the Golgi, given their apparent absence in other acidic organelles. The Golgi may also have a distinct V-ATPase that differs from other organellar V-ATPases by having a slightly different subunit composition or by being differently regulated. Misregulation of Golgi pH has already been shown to alter glycosylation in cancer cells and in cutis laxa, two disease states that do not seem to be related in any other way. While the defect in cutis laxa has been characterized, we currently do not know why Golgi pH is misregulated in cancer cells. Therefore, an important task in the near future is to resolve the underlying molecular defect(s) in cancer cells and to verify whether Golgi pH is also abnormal in cancer cells *in vivo*. This would then stimulate the development of novel diagnostic tools and glycosylation-based therapies to modulate cancer cell invasion and metastatic properties. Yet, the observations discussed above represent only the first examples of diseases that are caused by, or are associated with, misregulation of Golgi pH. Misregulation of Golgi pH may also play a role in cystic fibrosis and Ca<sup>2+</sup> pump-associated diseases. Other Golgi pH-associated diseases are likely to be found, and the search for these should be guided either by defects in Golgi structure or its glycosylation potential.

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