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Review Essay[1]

The function of the Golgi ribbon structure – An enduring mystery unfolds!

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Graphical abstractThe Golgi apparatus has recently emerged as a signalling hub for co-ordinating cellular processes. Here we explore the concept that the Golgi ribbon structure is critical in regulating higher order processes. Changes in Golgi ribbon morphology, i.e. fragmentation into mini-stacks, modulate cellular processes and are associated with diseases including neurodegeneration and cancer.

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Abstract
The Golgi apparatus in vertebrate cells consists of individual Golgi stacks fused together in a continuous ribbon structure. The ribbon structure *per se* is not required to mediate the classical functions of this organelle and the relevance of the "ribbon" structure has been a mystery since first identified ultrastructurally in the 1950s. Recent advances recognize a role for the Golgi apparatus in a range of cellular processes, some mediated by signalling networks which are regulated at the Golgi. Here we review the cellular processes and signalling events regulated by the Golgi apparatus and, in particular, explore an emerging theme that the ribbon structure of the Golgi contributes directly to the regulation of these higher order functions.

Abbreviations: TGN, *trans*-Golgi network; **GOLPH3,** Golgi phosphoprotein 3; **PI(4)P,** phosphatidylinositol-4-phosphate; **Aβ,** amyloid-beta peptides; **GRASP,** Golgi Reassembly Stacking Protein; **GM130,** *cis*-Golgi matrix protein; **Mena,** mammalian enabled; **GMAP210,** Golgi microtubule associated protein; **mTOR,** mechanistic target of rapamycin; **ERK,** extracellular signal-related kinase; **MAP,** mitogen-activated protein; **NMIIA,** non-muscle Myosin IIA; **Cdk5,** cell dependent kinase 5.

Keywords: cell proliferation; cytoskeleton; Golgi apparatus; golgins; Golgi ribbon; signalling; systems biology

1. Introduction

Analysis of the molecular networks over the past decade has provided deep insights into the organization of the cell as a highly regulated and integrated system. [1] There is an increasing appreciation that intracellular organelles have fundamental roles in co-ordinating vital cell processes, such as stress responses and apoptosis. The plasma membrane, endoplasmic reticulum and mitochondria are well known to function as sensors to regulate a variety of cell processes [2] in particular stress responses. However, the potential importance of the Golgi apparatus as a hub for monitoring and responding to cell processes had been largely overlooked until recently. Our historical understanding of the functions of the Golgi has been principally restricted to the regulation of glycosylation and membrane transport. Given the central location of the Golgi apparatus in the cell at the cross roads of the secretory and endocytic pathways, [3, 4] and the emerging principles of cellular networks, [1] it is very likely that the Golgi apparatus would contribute to the co-ordination of signalling and higher order cellular processes. Indeed, over the past few years considerable evidence has emerged that the Golgi apparatus feeds into the wiring of a range of cellular networks.

In protists, plants and invertebrates, individual mini-Golgi stacks are scattered throughout the cytoplasm of the cell. In contrast, vertebrates have evolved mechanisms for joining the individual Golgi stacks into a ribbon, typically found in a juxtanuclear location in interphase cells (Fig. 1). The organization of the Golgi apparatus is highly dynamic and the Golgi ribbon can dissociate and re-organise under a variety of conditions, for example during mitosis [5] and to reposition the Golgi to accommodate a number of processes, including directed secretion and pathogen invasion. [6, 7] Thus, there is considerable plasticity in the arrangement of the Golgi stacks and ribbon. Surprisingly, and despite our knowledge of Golgi dynamics, the fundamental biological question of the relevance of the "ribbon" structure of the Golgi in vertebrates has been explored in only a handful of studies. One reason for the slow progress is that the significance of the ribbon structure cannot be examined in the commonly used genetically amenable models of yeast and *Drosophila* as both these organisms have Golgi stacks which do not form ribbon structures, rather the isolated Golgi stacks in these organisms are scattered throughout the cytoplasm. Hence the study of the Golgi ribbon is restricted to vertebrate cells. Moreover, until recently the approaches for fragmenting the Golgi ribbon were reliant on the use of drugs such as nocodazole which is are relatively blunt and induce multiple cellular effect. More recent approaches have targeted structural proteins of the Golgi which has begun to reveal the complexity of pathways influenced by the Golgi apparatus and by the morphological status of this organelle.

It is now realized that intracellular organelles communicate with each other through membrane contacts. [8] The significance of these membrane contacts is only beginning to be appreciated. From a number of studies, it is clear that these membrane contacts control positioning of organelles, mobility of organelles mediated by the microtubule and actin cytoskeletons, lipid transfer and metabolic channelling. It is also possible that membrane contacts between intracellular organelles are important to integrate functions of different compartments. The Golgi has been shown to make contacts with the ER [9] and the late endosomes/lysosomes. [10] An issue that needs to be addressed is whether the morphology of the Golgi apparatus influences these intracellular network connections. This is relevant as the Golgi structure is the highly dynamic.

Based on cellular network principals and the recent identification of signalling molecules associated with the Golgi apparatus, [11, 12] here we review an emerging view that the Golgi apparatus in vertebrates contributes to the co-ordination of higher order cellular processes. In addition, we explore the concept that the ribbon structure of the Golgi plays an important role in the co-ordination of these higher order processes and that fragmentation of the Golgi ribbon into individual mini-stacks modulates and/or perturbs these processes.

2. Structure of the Golgi apparatus

The individual stacks of the Golgi apparatus are units of 4-8 flattened, cisternal membrane structures. [13] The *cis* and *trans* sides of these stacks are associated with networks of tubular structures – the *cis*-Golgi network or ER-Golgi intermediate compartment (ERGIC), and the *trans*-Golgi network (TGN) respectively, representing the cargo entry and exit points. The Golgi apparatus must maintain an ordered cisternal stack structure to ensure correct post-translational modifications of cargo and to promote efficient sorting and trafficking of secretory proteins. In vertebrate cells, the individual Golgi stacks come together to form a higher order structure [14]. Multiple Golgi stacks are linked together to form a reticular, twisted, ribbon structure that is actively maintained close to the centrosome by interactions with microtubules (Fig. 1A,B). [15] Golgi-derived microtubules have been proposed to draw individual stacks together, a process regulated by the microtubule-binding proteins to promote the formation of a continuous ribbon.[16, 17] In addition to microtubules, actin and actin-binding proteins participate in the structural organization of the Golgi and in trafficking processes. [18] The Golgi ribbon structure is very dynamic and can undergo profound remodelling events to reposition the Golgi during a number of processes. [7, 19] These remodelling events reflect dynamic interactions between Golgi membranes and the cytoskeleton, [4] as discussed further below. A number of Golgi "scaffold" proteins have been identified which link membranes to the cytoskeleton and regulate the Golgi ribbon structure and some of these scaffold molecules are also known to control signalling pathways, as discussed further in the following sections. Collectively, these Golgi scaffold molecules have the potential to be networked to a diverse set of cellular pathways.

3. The Golgi as a cell sensor

In addition to the classical roles of glycosylation and membrane transport, the Golgi apparatus has also been implicated in the regulation of a range of diverse functions including mitosis [20], apoptosis, [21] cell polarization ,[6] cell migration, [22] DNA repair, [23] metabolism, [24] microtubule organization [15] cell proliferation and signal transduction (Fig. 2). [11, 12]

A number of excellent recent reviews have summarized the signalling networks at the Golgi apparatus in general, and the reader is encouraged to source these reviews. [12, 22, 25, 26] Moreover, there has been considerable interest in the Golgi signalling pathways which regulate membrane transport of protein and lipid cargo along the anterograde route. [25-27] Here we will highlight the relationship between signalling and changes in the Golgi architecture, in particular signalling events associated with the fragmentation of the Golgi ribbon and regulation of higher order functions. Table 1 summarizes the cell processes and the Golgi-localized signalling pathways which influence or are influenced by the Golgi morphology. The following provides details of examples where such a relationship has been clearly demonstrated.

3.1 Signalling and the Golgi ribbon

Firstly, the Golgi ribbon is disassembled during mitosis into a collection of vesicles and tubules to allow partitioning of Golgi membranes between the two daughter cells. Mitotic disassembly of the Golgi ribbon is a very early event in G2/M transition, a characteristic of vertebrate cells, and considered to play an important role in promoting mitotic entry. [28] Prevention of ribbon disassembly, by interfering with the Golgi Reassembly Stacking Protein GRASP65 [29, 30] delays progression through the G2/M transition indicating that the change in Golgi morphology feeds into the cell cycle machinery pathway. Moreover, inhibition of the Golgi ribbon disassembly, by accumulation of a polymer of diaminobenzidene (DAB) in the Golgi lumen which inhibits membrane re-modelling, arrests cells in early mitosis. [31] The arrested cells lacked a bipolar spindle, had unseparated centrosomes, and had an active spindle assembly checkpoint. The mitotic block could be restored by centrosome depletion, indicating a centrosome-Golgi signalling axis which regulates the mitotic checkpoint [31]. However, the identity of these signalling components on the centrosome and Golgi are currently not known. In an unrelated study, a serine/threonine protein kinase (STK16) has been identified which is localized to the Golgi and regulates Golgi disassembly and assembly during the cell cycle by modulating actin dynamics .[32] Knock down of STK16 results in

delay in mitotic entry, [32] highlighting the role for a Golgi-localized protein kinase in cytokinesis. Collectively, there are multiple pathways of Golgi ribbon disassembly which actively regulate mitotic progression.

Secondly, cell polarization is critical for directional migration of cells in a variety of essential physiological processes, such as during development, in wound healing, and in metastasis. Also, polarized secretion is an essential feature of T lymphocytes and natural killer cells for effective immune responses. [33] These processes require directed trafficking of newly synthesized molecules to specific sites on the cell surface, for example the leading edge of the cell during migration or the immune synapse during immune responses. Polarized trafficking is achieved in many cells by the re-organization of the microtubule cytoskeleton and reorientation of the Golgi ribbon to a position ahead of the nucleus in the direction of either secretion and/or migration [17, 34]. In contrast, the arrangement differs in T cells, for example in activated cytotoxic T cells the centrosome, the major microtubule organizing centre, together with the Golgi apparatus are re-positioned close to the cell surface at the immune synapse on contact with the target cell [35]. A number of receptor signalling pathways mediate these cell polarization events. There is evidence that mitogen activated protein kinase (MAPK) signalling occurs at the Golgi and downstream targets include Golgi matrix proteins which regulate Golgi structure and function. [22] Hence, signalling events can result in re-organization of the ribbon structure to maximize delivery of proteins to specific domains on the cell surface.

Thirdly, the Golgi is also intimately associated with the biochemical pathways leading to cell death. The breakdown of the Golgi ribbon structure is an early event during apoptosis, mediated by caspase-cleavage of Golgi proteins. [21] Pathways connecting Golgi fragmentation and nuclear signaling have been suggested that involve caspase cleavage of golgins; namely golgin-160 by caspase 2, to release fragments which can be imported into the nucleus, [36, 37] and caspase cleavage of golgin p115 to release a 205 residue C-terminal fragment which is translocated into the nucleus and proposed to induce apoptosis by a p53-mediated pathway. [38]

Fourthly, the process of autophagy, which maintains cell homeostasis, involves the biogenesis of the “autophagosome” organelle to degrade proteins and alter the metabolism of cells. The source of membranes for initiating autophagy remains under debate; the Golgi, and

specifically the TGN, is considered to be a significant membrane source for the formation of newly forming autophagosomes. [39] Such processes would need to be tightly regulated by signaling events. Also, the Golgi has also been linked to pathways controlling metabolism and cell proliferation. [12, 24] For example, levels of phosphatidylinositol-4-phosphate (PI4P), regulated by the PI4 kinase (PI4K) and the 4-phosphatase, Sac1, controls anterograde trafficking as well as the structure of the organelle. [40] Sac1 levels at the Golgi are regulated by the metabolic state of the cell, indicating a molecular network between metabolism, signaling and Golgi morphology.

Fifthly, there is evidence of a network between glycosylation, Golgi structure, signalling at the cell surface and metabolism. Synthesis of highly branched glycans, mediated by Golgi localised glycosyltransferases, is a feature of vertebrate evolution. [41, 42] Galactose binding lectins, known as galectins, are exported from cells and cross link surface glycoproteins with highly branched glycans, forming a galectin-glycoprotein lattice which in turn regulates receptor levels and cell signalling. [43] Intriguingly, hexosamine flux and metabolism are linked with this pathway of glycan branching and galectin lattice formation. [42] Disruption of the Golgi ribbon structure, by depletion of GRASP65, or the *cis*-Golgi matrix protein GM130, has been shown to result in a perturbation in the distribution of glycosyltransferases involved in O-glycosylation across the Golgi stack and alterations in glycosylation of glycoproteins expressed at the cell surface. [44] Further, alterations in O-glycosylation and associated defects in galectin-mediated responses that regulate cell growth have been reported in tumour cells with fragmented Golgi ribbons. [45]

3.2 Signalling networks and Golgi morphology

A recent screen of signalling components has identified 180 signalling genes that have an impact on the organization of the mammalian Golgi. [11] This discovery strongly suggests a complex network of signalling pathways that regulate, and could be regulated by the Golgi structure. For example, it is now clearly established that constitutive transport processes are controlled by signalling pathways, a finding which represents a paradigm shift in the field. [46] Signalling pathways that have been associated with the Golgi include protein kinase C (PKC), protein kinase D (PKD), ras/MAP kinase, mTOR, and cAMP/protein kinase A (PKA) . (see [12, 25] Some of these signalling pathways, for example MAP kinase, also play a role in regulating the structure of the Golgi apparatus, a process mediated by scaffold molecules.

Recent bioinformatics analyses have identified multiple signalling networks which link stress response and DNA repair pathways to regulators of Golgi morphology. [47] Given the diverse range of signalling genes which have been identified to be wired to the Golgi apparatus, other cell processes in addition to those described above could also be influenced by Golgi signalling pathways.

4. Golgi proteins linking morphology, cytoskeleton and function

The Golgi ribbon morphology is maintained by interactions of a diverse range of Golgi proteins with the cytoskeleton. These include both membrane and peripheral Golgi proteins on the cytoplasmic surface of the Golgi membranes. The major classes of Golgi cytoskeletal interactive proteins are the coil-coiled domain golgins, [48] the stacking proteins GRASP55/65 [19] and the membrane tether Golgi phosphoprotein 3 (GOLPH3) . [49] GOLPH3 bridges the PI(4)P-rich TGN membrane and small actin filaments via the non-conventional motor MYO18A to induce actin-mediated tensile force. [50] There is also evidence that golgins can act as bridges to link both microtubules and actin cytosketelon to Golgi membranes, for example TGN golgin GCC185 interacts with the microtubule linker protein CLASP, [16] and golgin245/p230 has been reported to link both microtubules and actin. [4] Golgins can be considered to be scaffold molecules as they interact with a diverse set of binding partners including small G proteins, Arfs and Rabs, SNAREs, and cytoskeletal linker proteins. [4] Hence, golgins/membrane tethers represent potential molecular hubs that link Golgi morphology and cellular networks. Analysis of roles of the golgins in tissues and specialized cells by the expression of mutated forms of these molecules or by gene knock-outs, have revealed their importance in a broad range of cell and physiological processes. These findings could not have been predicted from the classical functions of the Golgi and are likely to reflect golgin-specific binding partners which integrate different molecular pathways in the cell. [51] For example, GMAP210 (Golgi microtubule associated protein) is a golgin localized at the *cis*-Golgi involved in the regulation of intra-Golgi transport and Golgi structure. [52] By RNAi, GMAP210 was shown to be required for the maintenance of the Golgi ribbon in cultured cells, although, depletion of GMAP210 did not affect the rate of secretory transport. [7, 53] A lack of GMAP210 is also associated with defects in ciliogenesis arising from the perturbation of Golgi structure which may reflect potential problems in re-positioning the MTOC (microtubule organising centre) and Golgi which is required for cilia formation. [54] Interesting, mice lacking GMAP210 have multiple phenotypes including skeletal, heart and

lung defects and die early in neonatal life. [55] Cells from these GMAP210 knock out mice have altered Golgi morphology and further studies are required to understand the molecular basis of the cellular pathways arising from the loss of the Golgi ribbon to account for these tissue specific defects *in vivo*.

Some pathways leading to Golgi fragmentation affect membrane transport, but many phenotypes associated with Golgi fragmentation have normal cargo transport. [56] Indeed, there are examples of specialized cells in vertebrates where the normal perinuclear Golgi ribbon structure is fragmented, such as differentiated myoblasts, [57] neurons which contain Golgi outposts along dendrites, [58] and gastric parietal cells. [59] We previously showed that the dispersed Golgi mini-stacks found in mature gastric parietal cells were functional in both anterograde and retrograde transport. [59] Hence, Golgi fragmentation can occur in normal development and the precise molecular pathway of Golgi fragmentation is likely to be important for the downstream functional consequence.

While the role of microtubules in defining the location and architecture of the Golgi in vertebrate cells is well appreciated, [60] the role of the actin-based cytoskeletal system at the Golgi has until recently been restricted to traditional Golgi functions associated with vesicle transport. [18] More recent studies have demonstrated a role for actin filaments in maintaining and in promoting fragmentation of the Golgi ribbon, and a growing list of actin regulators located throughout the Golgi have now been identified, which are summarized in Table 2. For example, the actin elongation factor Mena interacts with GRASP65 to enhance actin polymerization on individual Golgi stacks and then to pull the stacks together to promote ribbon formation. [61] In addition to the regulation of Golgi architecture, there are also examples where actin dynamics can influence signalling pathways. The Golgi structural protein, GOLPH3, mentioned above has been reported to connect Golgi membranes to actin cytoskeleton and drive an extension of the Golgi ribbon. [50] Given that MYO18A does not appear to have motor activity [62] it remains unclear at this stage how GOLPH3 binding partners provide the motor activity to generate a tensile force. Nonetheless, alterations in the levels of GOLPH3 protein at the Golgi influences not only Golgi morphology but also appears to modulate mTOR signalling and cell proliferation, [63] discussed further below. Another example is STK16, which directly interacts with actin and enhances actin polymerization. Both the actin binding activity, which controls Golgi structure, and the kinase

activity of STK16 is required for cell cycle progression. [32] Overall, these recent findings indicate that the ribbon morphology of the Golgi may be fine-tuned by machinery which regulates both, actin dynamics and Golgi ribbon structure.

Golgi fragmentation and loss of the ribbon structure has been investigated in a variety of pathological conditions. It is likely that the alteration in Golgi morphology in disease arises from the perturbation of a normal cellular process. For example, the dynamic balance between a ribbon structure and individual mini-stacks may be tightly regulated to modulate the wiring of networks via Golgi scaffold molecules. A key issue that now needs to be explored are the signalling pathways which are directly regulated by the different morphological states of the Golgi, i.e. ribbon versus mini-stacks.

5. Perturbations of Golgi ribbon in disorders and disease

The potential importance of the Golgi ribbon structure in regulating cellular processes is strengthened by numerous reports identifying major perturbations in Golgi morphology associated with disorders and diseases. These include stress responses arising from oxidative stress or infection, neurodegeneration, and cancer. From the discussion above, a plausible scenario is that the perturbation of the Golgi ribbon structure in these disorders/diseases contributes directly to the pathophysiological state.

A loss of the Golgi ribbon is observed in cells subjected to pharmacological and oxidative stress. [37] Fragments arising from cleavage of golgin 160 by caspase 2 accumulate in the nucleus and may influence gene expression and contribute to a stress repair pathway. [36] In addition, alcohol liver injury is linked to alterations in intracellular membranes and including remodelling and fragmentation of Golgi membranes, mediated by phosphorylation of non-muscle Myosin IIA (NMIIA) and reduced levels of the golgin giantin. [64]

5.1. Golgi fragmentation and neurodegeneration

The loss of the Golgi ribbon has been reported in a range of neurodegenerative diseases, [65] including Alzheimer's disease, Huntington disease, amyotrophic lateral sclerosis and Parkinson's disease, from both animal models and human disease. Whether Golgi fragmentation is contributing to the pathology of these diseases or is a consequence of other

cellular processes driving the pathology has been an unresolved question; however, a number of recent studies has strengthened the case that changes in Golgi morphology, and loss of the Golgi ribbon in particular, can directly cause neurotoxicity. The accumulation of amyloid beta (Aβ) in Alzheimer's disease has been reported to trigger Golgi fragmentation by activating cyclin dependent kinase 5 (cdk5) which phosphorylates the Golgi structural proteins GRASP65 and GM130 (*cis*-Golgi matrix protein) and causes Golgi fragmentation. [66] The loss of the Golgi ribbon in turn results in enhanced Aβ production and thereby promoting the production of the neurotoxic protein. [66] To directly assess whether perturbation of the Golgi structure results in neurodegeneration *in vivo*, the golgin GM130 was recently knocked out in neurons of mice and GM130 deficiency resulted in major changes in the Golgi architecture of primary neurons and neuronal loss. [67] These findings are significant as the data indicates that disruption of the Golgi ribbon, mediated by the knock-out of GM130, causes neurodegeneration. However, there are caveats to relating these findings to the human disease as there are many mechanisms/pathways which can result in Golgi ribbon disruption and the consequence of perturbing each of these pathways may be quite varied, even though they share the common feature of a disrupted Golgi. Knock-out of GM130 represents the removal of a major structural component of the Golgi, and the downstream consequence of this deficiency may not be similar to pathways associated with loss of Golgi ribbon in neurodegenerative diseases. A key question now is the identity of the relevant Golgi fragmentation pathways in these diseases and signalling network(s) which may be altered as a consequence of the Golgi morphological changes.

5.2. Cancer and Golgi morphology

A number of studies have recently reported a relationship between the Golgi ribbon integrity and transformation and cancer. The changes in Golgi morphology in tumour cells are associated with loss of normal polarity, defects in signalling mediated DNA repair pathways and galectin-1 induced apoptosis.

The Golgi structural protein, GOLPH3, has been identified as an oncogene. [63] Amplification or overexpression of the GOLPH3 gene results in Golgi fragmentation and enhanced cell survival after DNA damage [23]. On the other hand, depletion of GOLPH3 prevents dispersion of the Golgi (enhanced ribbon structure?) and increases apoptosis after DNA damage. [23] The nexus between Golgi morphology and DNA damage has been reported to be mediated by the

DNA damage response kinase, DNA-PK, which phosphorylates GOLPH3 and enhances its interaction with the actin cytoskeleton resulting in dispersal of Golgi fragments. GOLPH3 is also suggested to alter the response to mTOR signalling, [63] suggesting a relationship between Golgi morphology and mTOR signalling which needs to be further defined.

A recent report has demonstrated a relationship between the Golgi localised RhoGTPase signalling molecule RhoBTB1, Golgi structure and cancer cell invasion. The loss of Golgi localised RhoBTB1 in breast cancer cells reduced the expression of a Golgi arginine methyltransferase known as METTL7B and leads to Golgi fragmentation and loss of normal polarity. Re-expression of RhoBTB1 not only rescued normal Golgi morphology but also reduced tumour cell invasion. [68] Hence the perturbation of transcriptional targets by a Golgi localised small G protein alters Golgi ribbon structure and tumorigenesis.

In a study of prostate cancer, the Golgi was shown to be fragmented in androgen-refractory tumour cells whereas normal prostate cells have a typical compact Golgi morphology. The fragmented Golgi of the androgen-refractory tumour cells was associated with mislocalisation of a glycosyltransferase of the O-glycosylation pathway, resulting in altered O-glycosylation and defects in galectin-1-induced apoptosis. [69] The restoration of a compact "ribbon-like" Golgi morphology in these androgen-refractory prostate tumour cells, by knockdown of non-muscle myosin or treatment with drugs that inhibit actin polymerization, resulted in an increased susceptibility to galectin-1 mediated apoptosis, [69] indicating that the Golgi ribbon structure is regulating O-glycosylation pathways, [69] and glycan-galectin signalling pathways which influence proliferation and apoptosis. [43]

In summary, the Golgi responds to various signalling pathways by alterations in morphology and it is very likely that the Golgi apparatus can also transmit a range of signals, as illustrated by GOLPH3 mediated regulation of DNA repair. The morphology of the Golgi could well represent a mechanism for fine tuning signalling responses. For example, some signalling pathways are dampened as a consequence of the loss of the ribbon, for example p53-mediated apoptosis following DNA damage and cell migration, and some signalling pathways appear to be activated following Golgi fragmentation, such as the proposed Golgi-based G2/M checkpoint. [29, 70] Fig. 3 illustrates this concept. In this model, we propose that the balance of ribbon versus mini-stacks in the cell would define the qualitative and quantitative nature of a

range of signalling networks. The molecular mechanisms could involve scaffold molecules which interact with both actin regulators and signalling components; the recruitment of such scaffold molecules to the Golgi, or their dissociation by post-translational modifications, such as phosphorylation, would result in the re-modelling of the Golgi structure via the actin cytoskeleton and also define the strength of signalling of a given pathway. The capacity to fine tune signalling pathways is fundamental in developmental and physiological processes, and the evolution of the Golgi ribbon in higher eucaryotes may have provided an additional structural platform to enhance the regulation of complex systems compared with lower eukaryotic organisms. Although non-vertebrates such as flies and worms lack a Golgi ribbon and have many of the basic processes described in the review, the evolution of higher eukaryotes is intimately associated with additional sophisticated regulatory networks. We propose that the Golgi ribbon represents an additional structural entity in higher vertebrates to finely tune signalling and cellular networks.

6. Way forward: A Systems approach to reveal the role of Golgi ribbon in cell pathways

Many of the approaches previously used to study the functional role of the Golgi ribbon have been very non-specific, for example treatment with the microtubule depolymerising drug nocodazole. [71] Ablating Golgi components which are required for membrane transport can also result in loss of the ribbon and dispersal of individual stacks, [44, 72] however, the knock down of such components is likely to result in multiple effects. Perturbation of the levels of the Golgi scaffold molecules, discussed in this review, provides a more sophisticated approach to analyse the function of the Golgi ribbon structure and such systems now need to be developed further.

The following experimental strategies would be instructive to provide a better understanding of the function of the ribbon structure of the Golgi in higher order functions in vertebrate cells. First, one of the challenges in understanding the relationship between the form and function of the Golgi apparatus is that altered morphology of the Golgi is often defined under the common term of "Golgi fragmentation" to depict the perturbation of Golgi ribbon as revealed by optical microscopy. However, the structures of the Golgi fragments are likely to differ depending on the nature of pathway involved to perturb/disrupt the Golgi ribbon. It is clear that there are many different pathways result in "Golgi fragmentation" and the different pathways are associated with different cellular outcomes. Ultrastructural analysis is required

to define the nature of the Golgi elements in each of these different pathways i.e. are they isolated Golgi mini-stacks or do they represent membrane fragments of different cisternae, as the functional outcome is likely to be very different depending on the precise Golgi structures.

Second, defined cell-based systems need to be developed to analyse the function of the Golgi ribbon structure. One approach worth exploring is to modulate the Golgi ribbon by a modest overexpression and knock down of membrane scaffolds that influence the tensile force of the actin cytoskeleton. By developing inducible expression systems, the Golgi ribbon could be modified in a predictable, defined and reversible manner. Such a system would be applicable to not only immortalized cell lines but also, and most importantly, to primary cells and the whole organism so that the Golgi ribbon could be analysed within selected specialised cells during development and in the adult.

Thirdly, to fully appreciate how the Golgi ribbon structure in vertebrates contributes to the co-ordination of higher order cellular functions, an unbiased system biology approach should be employed using cells that can be modulated between the Golgi ribbon and Golgi mini-stacks morphology. For example, next generation sequencing such as RNA seq, protein microarray, and central carbon metabolism analyses will allow global changes in cellular processes to be defined between cells with Golgi ribbons and Golgi mini-stacks. In addition, comprehensive analyses of phosphorylated signalling components by phosphoproteomics could identify alterations in signalling pathways as the Golgi ribbon is either formed or dismantled. Such data sets would provide a global view of signalling pathways that are regulated by the Golgi ribbon structure and the cell processes which are controlled by the Golgi morphology (Fig. 3). Such an approach should provide invaluable information for understanding the consequence of phenotypic alterations of the Golgi apparatus associated with a variety of conditions and diseases.

7. Conclusion

Since first discovered by Camillo Golgi in 1898, the Golgi apparatus has both intrigued and challenged cell biologists to understand the relationship between the form and function of this organelle. The role of the Golgi in the regulation of membrane transport and glycosylation has dominated the Golgi field for the past 50 years; advances of the past 5-10 years have revealed that the Golgi apparatus also regulates a diverse range higher order functions. Fragmentation of the Golgi ribbon structure is closely associated with alterations in many cellular processes.

A number of signalling components have been identified at the Golgi apparatus. The challenge now is to map the complete suite of signalling pathways at the Golgi that are wired into cellular processes and to determine how the changes in the dynamic ribbon morphology of the Golgi regulates these networks. The molecular tools and technologies are now available to manipulate the balance between the ribbon structure and Golgi mini-stacks and to apply a systems approach to understand how the ribbon structure of the Golgi regulates cell development, metabolism and proliferation in specialized cells in the whole organism and why the loss of the Golgi ribbon structure is associated with a variety of pathological conditions.

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FIGURE LEGENDS

Figure 1. The structure of the Golgi apparatus in different organisms. A: Golgi mini-stacks are dispersed throughout the cytoplasm in yeast, plants, and invertebrates. **B:** In vertebrates, the Golgi is organised as a ribbon structure in the perinuclear region of most cells maintained close to the centrosome (small red oval) by interactions with microtubules (blue).

C: Structured illumination super-resolution microscopy of HeLa cells stained for a cis-Golgi marker (red) and a TGN marker (green) which illustrates the continuous ribbon structure of the Golgi. Scale bar represents 5 μm.

Figure 2. Proposed higher-order functions that may be regulated by the ribbon structure of the Golgi apparatus.

Figure 3. A model for the structure of the Golgi regulating signalling pathways. The model proposes that a variety of signalling inputs are received by the Golgi and a variety of signal pathways are transmitted from the Golgi. Based on the discussion in the text, the model proposes that the identity and activation of these signalling pathways depends on the organization of the Golgi structure. Thickness of arrow indicates signal strength. Differences in strengths of signalling pathways are proposed to be associated with the Golgi ribbon or with Golgi mini-stacks. Individual signalling pathways are indicated by different colours.

TABLE 1

Signal-mediated pathways which influence or are influenced by the Golgi structure[1]

Processes/ Pathways	**Regulators of signalling pathways at the Golgi**	**Alterations in the Golgi architecture**	**References**
Apoptosis	p115 (fragments)	Cleavage of p115 at the *cis*-Golgi by caspases 3 and 8 leads to Golgi fragmentation	[38, 73]
Apoptosis	golgin-160 (fragments)	golgin-160 is cleaved by caspases 2, 3, and 7 leading to Golgi fragmentation	[74]
Cell proliferation/ mTOR signalling	PI(4)P / GOLPH3	Overexpression of GOLPH3 causes Golgi fragmentation and an increase in mTOR activity in cancer cells.	[63]
Autophagy	Bif-1/Atg9	Golgi localised Bif-1 regulates the fragmentation of Atg9-positive Golgi membranes during starvation, thus initiating autophagosome formation.	[75]
Cell polarization/MAPK signalling	ERK/ MAP kinases	ERK phosphorylates GRASP65 which promotes re-modelling of Golgi architecture for establishing polarization of Golgi structures in migrating cells; promote Golgi disassembly during mitosis	[34]

Cell migration/MAPK	JNKs	Knockdown of JNK2, JNK3 and p38alpha MAPK disrupts Golgi architecture and inhibits cell migration	[76]
Cell migration/ERK signalling	p190RasGAP	Cells lacking p190RasGAP exhibit higher levels of active ERK resulting in Golgi fragmentation, which perturbs directional cell migration.	[77]
Golgi-to-ER retrograde transport	Protein Kinase A (PKA)/cAMP	Depletion of PKA regulatory subunits using RNAi or inhibition of PKA with specific drugs induces fragmentation of the Golgi; in contrast, stimulation with cAMP elicits fusion and condensation of Golgi cisternae to more compact structures.	[78]
N-glycosylation /trafficking	Hck (Src kinase family member)	HIV Nef mediated activation of Hck at the Golgi promotes serine phosphorylation of GRASP65, inducing Golgi cisternal unstacking.	[79]
Mitosis	ERK/MEK1	A Golgi localised isoform of ERK1, ERK1c, mediates Golgi fragmentation and is essential for mitotic progression.	[80]
Cell polarization/ Centrosome-Golgi axis	CG-NAP/ AKAP450	cis-Golgi localised CG-NAP/AKAP450 interacts with and recruits PKN, PP2A, or PP1to the Golgi and may influence various signal transduction pathways. Also essential for Golgi-microtubule nucleation and positioning of the Golgi ribbon near the centrosomes.	[81]

[1]Abbreviations: mTOR, Mechanistic Target Of Rapamycin; Bif-1, Bar Interacting Factor 1; Atg9, autophagy related protein 9; ERK, extracellular signal-related kinase; MAP, mitogen-activated protein; JNK, c-JUN N-terminal kinase; GAP, GTPase activating protein; Hck, Hematopoietic Cell Kinase; MEK1, MAP/ERK; CG-NAP, centrosome and Golgi localized PKN-associated protein; AKAP450. A-kinase anchoring protein 450; PKN, serine/threonine-protein *kinase* N1; PP2A, protein phosphatase 2A; PP2A, protein phosphatase 2

TABLE 2

Golgi scaffold/linker molecules which interact with the actin cytoskeleton

and influence Golgi structure

Scaffold Molecule	Localisation and interactive partners	Impact of overexpression and/or depletion	References
GOLPH3	*trans*-Golgi protein, recruited to membranes by interaction with PI4P. Interacts with MYO18A	Depletion of GOLPH3 causes Golgi compaction. Overexpression leads to fragmentation of Golgi	[50, 82]
GRASP65	*cis*-Golgi golgin. Interacts with Mena to promote actin polymerization	GRASP65 deletion causes defects in Golgi ribbon and fragmentation of ribbon	[61]
Giantin	Type II membrane matrix protein reported to negatively regulated actin dynamics by regulating the interaction of non-muscle myosin IIA (NMIIA) heavy chain with Golgi membranes and actin	Reduced levels of giantin results in Golgi fragmentation	[64, 83]
GM130	cis-Golgi matrix protein reported to recruit the GEF (Tuba) for Cdc42 activation and actin polymerization.	Depletion of GM130 reduced steady state levels of Cdc42 and results in Golgi fragmentation	[45]
STK16	A myristolylated Golgi serine/threonine kinase which directly binds actin and regulates actin polymerization.	Knock down results in fragmented Golgi	[32]
Golgin245/p230	Interacts with MACF1 (Microtubule-Actin Crosslinking Factor 1) protein (>600 kDa) that links microtubules to the actin cytoskeleton	Depletion results in fragmentation and dispersal of Golgi stacks	[84] [85]
Optineurin	A golgin, which links myosin VI to the Golgi complex	Depletion of optineurin by RNAi results in fragmentation of Golgi ribbon	[86]
Intersectin 1 (ITSN)-Cdc42 complex	ITSN1 has been suggested to be located to Golgi. It is a GEF for Cdc42, a Rho GTPase family member, which promotes actin polymerization.	A small molecule inhibitor which blocks ITSN1-Cdc42 interaction disrupts Golgi organisation	[87]

FH2 domain-containing protein 1 (FHDC1)	A Golgi localised formin which binds actin via its FH2 domain	Overexpression disperses the Golgi ribbon into functional ministacks	[88]

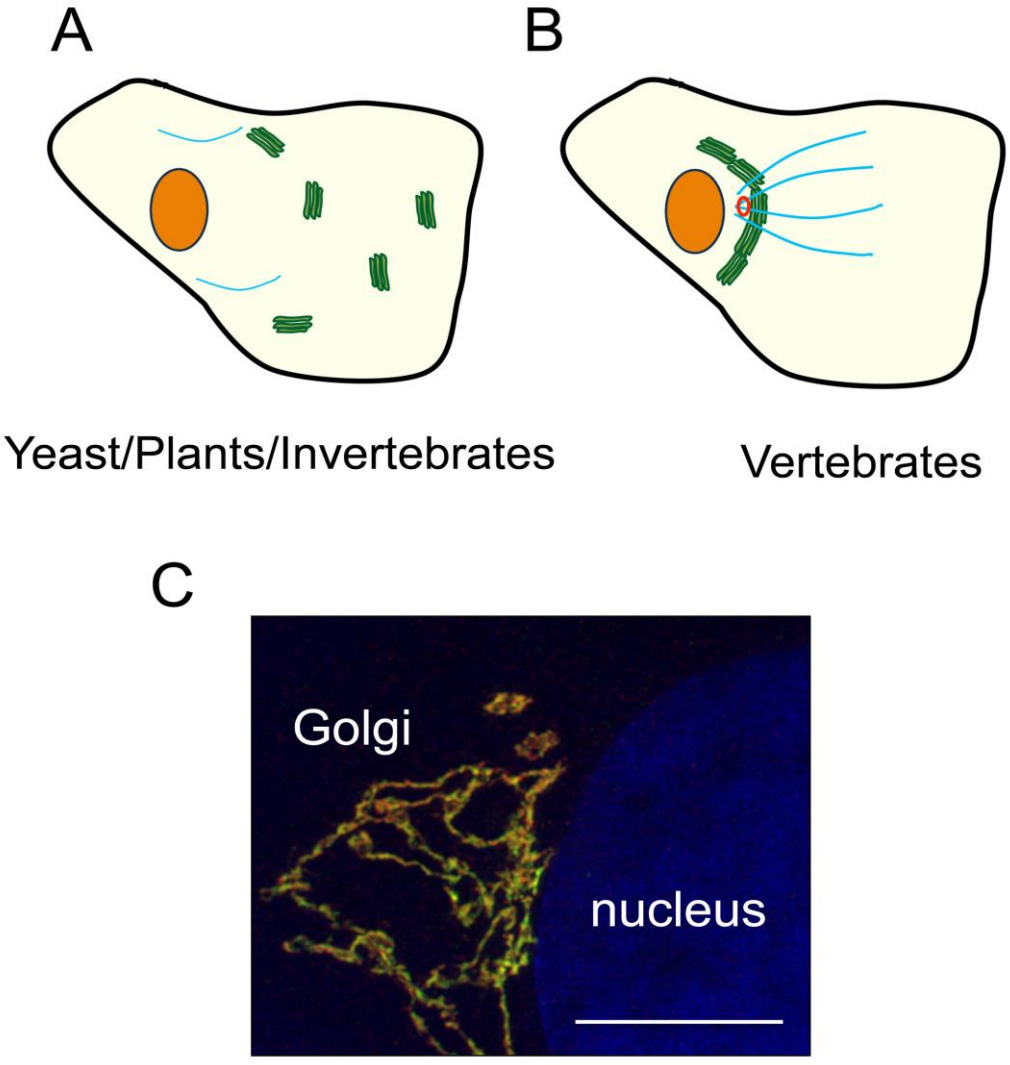

Figure 1

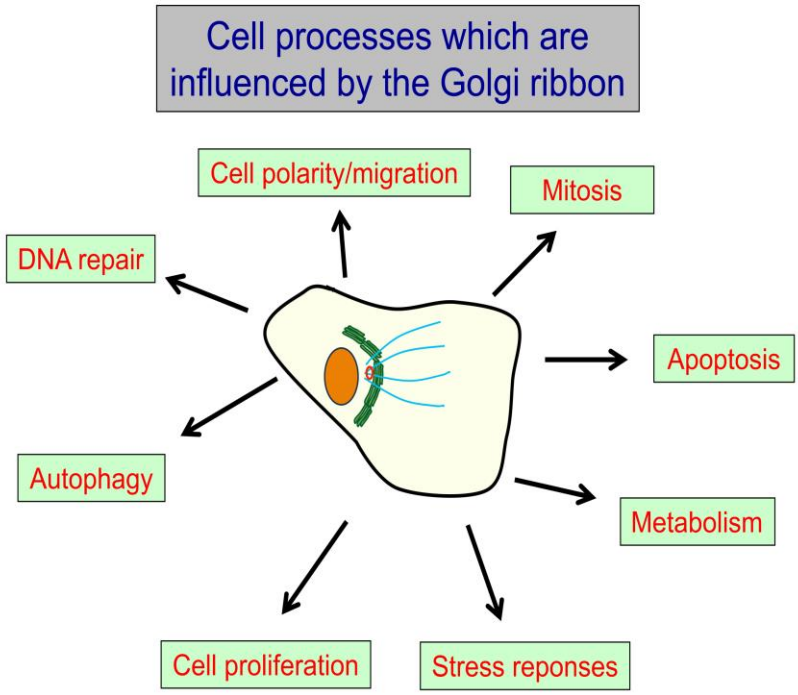

Figure 2

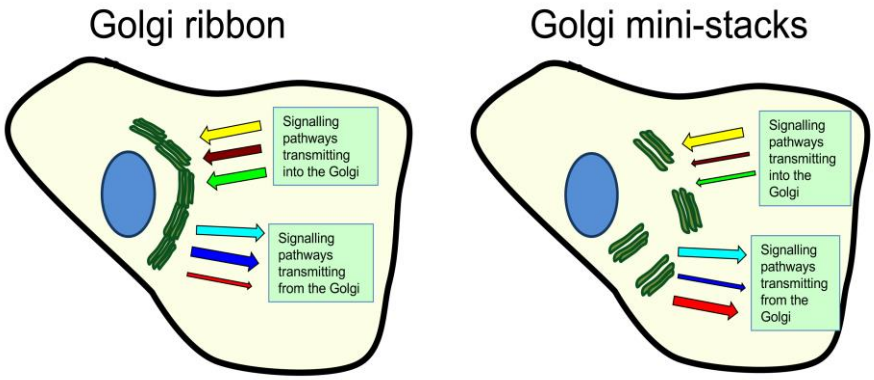

Figure 3