Review

Golgins and GTPases, giving identity and structure to the Golgi apparatus

Benjamin Short, Alexander Haas, Francis A. Barr*

Intracellular Protein Transport, Independent Junior Research Group, Max-Planck-Institute of Biochemistry, Martinsried, 82152, Germany

Received 3 November 2004; received in revised form 9 February 2005; accepted 9 February 2005
Available online 16 March 2005

Abstract

In this review we will focus on the recent advances in how coiled-coil proteins of the golgin family give identity and structure to the Golgi apparatus in animal cells. A number of recent studies reveal a common theme for the targeting of golgins containing the ARL-binding GRIP domain, and the related ARF-binding GRAB domain. Similarly, other golgins such as the vesicle tethering factor p115 and Bicaudal-D are targeted by the Rab GTPases, Rab1 and Rab6, respectively. Together golgins and their regulatory GTPases form a complex network, commonly known as the Golgi matrix, which organizes Golgi membranes and regulates membrane trafficking.

Keywords: Golgin; Rab; ARF-like; ADP-ribosylation factor; GTPase; Golgi matrix

1. The Golgi apparatus, together apart

The Golgi apparatus is a series of flattened, cisternal membrane structures forming the heart of the secretory pathway. Strikingly, in most animal and plant cells these cisternae, unlike the endoplasmic reticulum, do not fuse into one continuous reticular structure, but are closely juxtaposed in a stack-like organization. This raises the obvious question of how cisternae are held closed together in stacks, but do not fuse, while at the same time vesicles that are tethered to these stacks can fuse with the Golgi cisternae. In animal cells, these stacks are then arranged end to end to form the ‘Golgi ribbon’ [1]. The Golgi stack is a polarized structure with a cis-face exchanging proteins and lipids with the endoplasmic reticulum (ER), and a trans-face communicating with the plasma membrane and compartments of the endocytic pathway. Complex networks are found at each of these faces, referred to as the cis-Golgi network (CGN) and the trans-Golgi network (TGN) [2,3], respectively. As secretory material passes through the Golgi in a cis to trans fashion, it becomes post-translationally modified in a sequential order before being sorted at the TGN for delivery to its final destination within the cell. Despite this highly organized structure the Golgi is a highly dynamic organelle. For example, it is estimated that, in the exocrine pancreas, more secretory material enters the Golgi from the ER every 5 min than there is protein in the Golgi [1]. Nevertheless, the Golgi manages to maintain its high degree of structural organization and, indeed, must do so in order to ensure that secretory proteins are correctly modified and sorted. Many Golgi-resident proteins are, therefore, involved in forming a matrix that helps maintain the structure of this highly dynamic organelle.

One of the first clues that a subset of Golgi proteins played such a role came from the electron microscopy studies, which identified proteinaceous bridges linking adjacent cisternae together [4,5]. This was followed by the demonstration that isolated Golgi membranes retaining their stacked cisternal structure could be detergent-extracted to leave a proteinaceous exoskeleton with the characteristic organization of Golgi cisternae that is capable of binding Golgi resident glycosyltransferases [6].

More recently, the components of the Golgi matrix have begun to be characterized at a molecular level. An important feature of many but not all of these components is that they

* Corresponding author. Present address: Laboratory of Mammalian Cell Biology and Development, The Rockefeller University, 1230 York Avenue Box #300, New York, NY 10021, USA. Tel.: +49 89 8578 3135; fax: +49 89 8578 3102.
E-mail address: barr@biochem.mpg.de (F.A. Barr).
behave differently to Golgi-resident enzymes when cells are treated for short periods with reagents blocking ER-to-Golgi transport (for example, with the fungal metabolite brefeldin A (BFA) or dominant-negative forms of the COPII vesicle coat component Sar1p). Under these conditions, many Golgi resident proteins relocalize to the ER [7], yet some Golgi-like structures containing components of the Golgi matrix remain distinct from the ER [8]. Principally, these are members of the golgin family of Golgi-localized coiled-coil proteins and the GRASP family of Golgi stacking proteins. As we shall see, it is a network of interactions between these proteins, and their regulation by small GTPases of the Rab, ADP-ribosylation factor (ARF), and ARF-like (ARL) families that is responsible for the dynamic organization of the Golgi apparatus. This review will focus on the molecular characteristics of the golgin family of proteins and their regulation by small GTPases, other aspects such as Golgi biogenesis have been reviewed elsewhere [9,10].

2. Golgins: a diverse family of coiled-coil proteins

The golgins were originally identified as a family of Golgi-localized autoantigens, using antibodies derived from the sera of patients with a variety of autoimmune disorders [11]. All contain large regions of coiled-coil, a common protein motif known to form an extended rod-like structure [12]. Exactly why golgins are autoantigens is still unclear, but autoantibodies to them may well arise as a result of the breakdown of the Golgi during apoptosis and necrosis leading to the generation of multiple antigenic fragments from the repetitive coiled-coil domains [13].

More recently, several additional examples of coiled-coil proteins localizing to the Golgi apparatus have been found. Although no autoantibodies to these proteins have been found so far, they have also been classified as golgins due to their extensive coiled-coil structures [14,15]. Examples of the different types of golgin and a summary of the mechanisms they used to target to the Golgi are shown in Fig. 1, and discussed later in this review.

Apart from Golgi localization and the presence of coiled-coil motifs, the only other common feature so far identified in the golgin family is that many members interact with small GTPases. This diversity amongst the golgins is reflected in the wide range of functions that they carry out. Indeed, the family is so diverse in both structure and function that different organisms appear to have different complements of golgins in their genome. For example,
While p115 can be found in all eukaryotes and GRASPs are found in all eukaryotes except plants, golgin-45 is present only in vertebrates, and GM130 is present only in mammals. This doesn’t mean that they are unimportant. Rather, it probably indicates that the Golgi is structurally adapted in different species to meet the specific needs of different cell types. For example, the recent finding that GM130 is not required for mammalian cell viability at 34.5 °C, but is essential at higher temperatures could indicate that this golgin has arisen in response to the requirements of growth at higher body temperatures. It is interesting to speculate that organisms which typically live at an ambient temperature of between 4 and 20 °C such as nematodes and yeasts may therefore get away with a simpler complement of golgins, and thus not require a GM130 homologue. The modular structure of golgins, with central coiled-coil regions and functional domains at the N- and C-termini, is thus ideal for such wide adaptations. A similar situation exists with the cognate GTPases regulating particular golgins, Rab1 like p115 is found in all eukaryotes, while Rab2, like its partner golgin-45, is also only present in vertebrates.

One of the best-characterized functions of golgins is their role in membrane tethering events, as exemplified by the cis-Golgi golgins, p115, GM130 and giantin (Fig. 2a–c). The tethering factor p115 was originally identified using the Rothman intra-Golgi transport assay [16]. It is a peripheral membrane protein mainly localized to the vesicular-tubular clusters of ER to Golgi transport intermediates (VTCs) and the cis-Golgi [16,17] and was shown by rotary shadowing to be a homodimer with an N-terminal globular head domain and a C-terminal coiled-coil domain with a length of 45 nm [18]. A short acidic patch is located at the extreme C-terminus of the protein. The budding yeast homologue of p115, Uso1p, is similar in structure but is much larger in size (approximately 150 nm) [19]. Studies on Uso1p provided insight into its function as a tethering factor. Along with the small GTPase Ypt1p (yeast Rab1), it is required for the docking of ER-derived COPI-coated vesicles with Golgi membranes in an in vitro assay [20,21]. Recent studies on mammalian p115 have suggested a direct interaction with SNARE proteins that catalyze membrane fusion. Not only could p115 bind to certain early Golgi SNARE proteins but an in vitro assay showed that it could actually stimulate SNARE complex assembly, thereby linking initial membrane tethering to fusion [22]. Additionally, it has been shown that p115 is important for COPI, as well as COP II, vesicle docking [23]. To accomplish these functions, p115 interacts with two additional golgins at the cis-Golgi: GM130 and giantin.

GM130 was originally identified (as golgin-95) using antisera from an autoimmune disease patient and, subsequently, by raising antisera against solubilised Golgi matrix [24,25]. Localized to the cis-Golgi, GM130 is predicted to contain extensive regions of coiled-coil forming a homodimer, and has a basic domain of 75 amino acids at its N-terminus, which binds to the C-terminal acidic patch of p115 [26]. GM130 is targeted to cis-Golgi membranes by its tight binding to GRASP65, an interaction that requires a motif at the extreme C-terminus of GM130 [27].

Giantin, on the other hand, is an integral membrane protein of 400 kDa, with a C-terminal transmembrane domain and a very large cytoplasmic domain containing extensive regions predicted to form coiled-coil [28]. It is localized to the edges of the Golgi stack and on COP I vesicles and also binds p115 via its N-terminus [23].

How do p115, GM130, and giantin act together to mediate docking events at the cis-Golgi? The presence of giantin in COPI vesicles and GM130 on Golgi membranes, and the ability of both proteins to bind p115, suggest a model in which COPI vesicles are linked to their target membranes by a giantin–p115–GM130 ternary complex [23]—although this wouldn’t apply to ER-targeted COPI vesicles. Such a model is supported by an in vitro assay in which the docking of COPI vesicles with Golgi membranes is inhibited if the vesicles are pre-incubated with anti-giantin antibody but is unaffected if the Golgi membranes are pre-incubated with anti-giantin. Anti-GM130 antibodies, on the other hand, only inhibit the assay if they are pre-incubated with Golgi membranes but have no effect when pre-incubated with COPI vesicles. The same assay shows a dependence on p115 since addition of increasing amounts of the protein increases the rate of vesicle docking [23].

The idea of a giantin–p115–GM130 ternary complex is contradicted however by evidence that GM130 and giantin compete for the same binding site on p115 [29]. Also, studies on trafficking between the ER and the Golgi suggest that the three proteins act at kinetically distinct stages since anti-p115 antibodies inhibit transport at the VTC stage while anti-GM130 and anti-giantin inhibition occurs at the Golgi—with giantin inhibition occurring later than GM130 inhibition [30].

Since p115, GM130, and giantin are implicated in such a variety of different processes (including both retrograde and anterograde transport steps as well as cisternal stacking), it is possible that they act in discrete ways during different events. The effects of antibody inhibition may vary depending on precisely which process is being measured in a particular assay. In any case, the interactions of these golgins, both with each other and with other proteins, are clearly important for vesicle tethering events at the cis-Golgi. Additionally, these three golgins have also been implicated in the stacking of Golgi cisternae when the Golgi reforms following mitosis, a case of membrane tethering without subsequent fusion [31]. The complex formed by p115, GM130, and giantin may therefore be the proteinaceous material seen linking Golgi cisternae as well as the ‘strings’ seen between tethered vesicles and their target membranes in electron micrographs [5,32].

Recently, the question of whether GM130 is essential for maintaining Golgi structure has been investigated in a conditional lethal mutant cell line, ldlG, which lacks detectable levels of GM130 protein [33]. The fact that, at
higher temperatures, these cells have a disrupted Golgi, show defects in secretion, and eventually die demonstrates that, indeed, GM130 is essential for normal Golgi structure and function. On the other hand, ldlG cells do manage to maintain a normal Golgi at the lower temperature of 34.5°C, indicating that other proteins are able to compensate for the absence of GM130 under these conditions [33].

While GM130 and its interaction partners may control the formation of the Golgi stack, another golgin, golgin-84, appears to regulate Golgi structure at a different level—namely the lateral organization of stacks into the Golgi ribbon [34,35]. Primarily localized to CGN, golgin-84 is an integral membrane protein that interacts with the GTPase Rab1 [34,35]. The transmembrane region of golgin-84 can catalyse this event, although the mechanism is unclear. (d) Recruitment of the dynein–dynactin complex to vesicles by Rab6. Activated Rab6 recruits the dynein motor to Golgi membranes and transports vesicles via multiple interactions with the dynactin complex and the golgin Bicaudal-D. Bicaudal-D acts as an attachment factor for the dynactin complex, and binds Rab6 via its C-terminal domain. In addition Rab6 interacts with the dynacin subunit p150glued. We speculate that dynacin at microtubule tips can capture Rab6 bearing Golgi vesicles, and that dynein then transports these membranes towards the microtubule minus end. See the text for more details and references. Diagram is not to scale.

Fig. 2. Membrane tethering systems at the Golgi. The role of the p115 the tethering system in ER to Golgi, and intra-Golgi traffic. (a) COPII vesicles formed at ER exit sites are clustered together by the action of the golgin tethering factor p115. Rab1 recruits p115 on to these vesicles by interactions with the globular head and neck regions of p115. These tethered vesicles then fuse in an NSF and SNARE dependent mechanism to form VTCs. (b) VTCs then dock with the cis-Golgi, this event is thought to be facilitated by an interaction between the Rab1–p115 complex on the VTCs and GM130–GRASP65 on the cis-Golgi. An acidic C-terminal patch on p115 directly binds to a complementary basic patch at the N-terminus of GM130. (c) p115 and GM130 may also play additional roles in docking COPI vesicles to the cis-Golgi. This event is mediated via an interaction of the transmembrane golgin giantin on COPI vesicles with GM130 at the cis-Golgi. p115 can catalyse this event, although the mechanism is unclear. (d) Recruitment of the dynein–dynactin complex to vesicles by Rab6. Activated Rab6 recruits the dynein motor to Golgi membranes and transports vesicles via multiple interactions with the dynactin complex and the golgin Bicaudal-D.

B. Short et al. / Biochimica et Biophysica Acta 1744 (2005) 383–395
binding domain of golgin-84 to an in vitro Golgi reassembly assay promoted the lateral growth of the Golgi cisternae [35]. Depletion also resulted in an increase in the amount of ER membrane relative to the Golgi membrane, and a two-fold reduction in transport from the ER to the cell surface [34]. This latter result implies that p115/GM130/giantin are not the only golgins regulating ER-to-Golgi transport. Diao et al. [34] proposed that while p115, GM130, and giantin have a direct role in ER-to-Golgi transport, golgin-84 regulates formation of the Golgi ribbon. The depletion of golgin-84 might then inhibit protein transport if trafficking occurs less efficiently through individual Golgi stacks than through a fully formed ribbon.

Other golgins perform different roles that contribute to efficient transport at the Golgi. The two related golgins Bicaudal-D1 and Bicaudal-D2 have recently been shown to localize to the trans-Golgi through their interaction with Rab6 [37,38]. In addition, the Bicaudal-D proteins interact with dynactin, an adaptor for the microtubule-based, minus-end directed motor protein dynein [39]. Given that the Golgi apparatus is positioned in a dynein-dependent manner in the pericentriolar region of animal cells [40,41], and that Rab6 controls microtubule-dependent retrograde transport from endosomes to the trans-Golgi and from the Golgi to ER [42–44], Rab6 and Bicaudal-D probably combine to tether transport vesicles directed toward the Golgi to the cytoskeleton and collect them in the pericentriolar region. Thus, rather than tethering membranes together, Bicaudal-D is a golgin that tethers vesicles and possibly some Golgi membranes to the microtubule cytoskeleton.

3. GRASP proteins stack the Golgi together

A second important component of the Golgi matrix is a family of proteins identified using a functional assay for the post-mitotic reassembly of Golgi stacks. The inhibition of this assay by the alkylation agent NEM allowed the identification of two GRASPs (for Golgi Reassembly Stacking Proteins), with molecular weights of 55 and 65 kDa [45,46]. GRASP65 is localized to the cis-Golgi while GRASP55 is predominantly at the medial Golgi. Both proteins are peripheral membrane proteins on the cytosolic face of Golgi cisternae, and are associated with the membrane by N-terminal myristoylation (although how the proteins are targeted specifically to Golgi membranes remains unclear). Both proteins are phosphorylated during mitosis, which may be significant for the mitotic disassembly of the Golgi apparatus. Their role in the reassembly of the Golgi following mitosis was made clear by experiments in which blocking antibodies specific for either of the GRASPs, or non-myristoylated (i.e. non-membrane targeting) versions of the proteins could inhibit cisternal stacking in an in vitro assay [45,46]. More recently, the microinjection of anti-GRASP65 antibody into semi-permeabilised cells was shown to impair the breakdown of the Golgi apparatus induced by mitotic cytosol and, indeed, microinjection into intact cells prevented mitotic entry, suggesting a ‘Golgi checkpoint’ in which fragmentation of the Golgi is required for the cells to enter mitosis [47]. This effect is mediated through the polo-like kinase (Plk1; see [48] for a review of Plk1 function), which docks to the C-terminal domain of GRASP65 once it has been phosphorylated by Cdk1–cyclin B [49]. At present it is unknown what the downstream targets of Plk1 are in this context.

One important way in which the GRASP proteins regulate Golgi structure is likely to be their interactions with members of the golgin family. GRASP65 is an adaptor for GM130, and this interaction is necessary for both proteins to localize to Golgi membranes [27]. Similarly, GRASP55 is a specific binding partner of the medial-Golgi localized golgin-45. Disruption of this complex by the depletion of golgin-45 results in the dispersal of the Golgi apparatus and inhibition of protein transport [50]. In addition, GRASP65 has been shown to interact with members of the p24 family of cargo receptors involved in recycling between the ER and Golgi [51] and GRASP55 binds to Transforming Growth Factor-α (TGF-α), an interaction important for TGF-α’s expression at the cell surface [52]. Thus, GRASP proteins and their binding partners are important for both Golgi structure and function, providing a link between the Golgi matrix and certain integral membrane cargo proteins.

4. Dynamics of the Golgi matrix

As discussed above, the Golgi matrix must be a highly dynamic structure in order to facilitate the constant remodelling of the Golgi apparatus as it fulfils its function. Components of the matrix interact with Golgi membranes in a variety of ways. Some golgins are integral membrane proteins but many are peripherally associated, potentially allowing their rapid recycling from one part of the Golgi membrane to another via the cytosol. The variety of ways in which golgins associate with Golgi membranes is illustrated in Fig. 1.

The GM130–GRASP65 complex associates with high affinity to Golgi membranes through the myristoyl group of GRASP65 [27,45]. However, photobleaching experiments using green fluorescent protein (GFP)-tagged proteins suggests that the complex undergoes rapid recycling between the Golgi membranes and the cytosol [7]. It is unclear what the receptor for the GM130–GRASP65 complex is on Golgi membranes and how the association and disassociation with this receptor is regulated. In principal, the myristoyl group of GRASP65 could associate with any membrane and yet newly-synthesized GRASP65 associates directly with the cis-Golgi and not with any other membranes such as the ER [53]. One possibility is that GRASP65 recognizes Golgi membranes by binding to the cytoplasmic domains of transmembrane proteins such as the
p24 family [51]. GRASP65 is able to distinguish between the different oligomeric states of the p24 proteins and might therefore be able to determine its specific localization to Golgi membranes in vivo by only binding the Golgi-localized pool of p24 proteins, which are thought to exist in a higher oligomeric state than the ER-localized pool [51, 54].

The localization of other peripheral membrane golgins to Golgi membranes is dependent upon their interactions with small GTPases such as the Rab proteins (see below). These GTPases themselves cycle between cytosolic and membrane-bound states in a nucleotide-dependent manner and are subject to regulation by accessory factors such as specific GTPase Activating Proteins (GAPs) and Guanine nucleotide Exchange Factors (GEFs). Membrane-associated, GTP-bound (active) Rabs are then able to recruit golgins to the membrane.

Integral membrane golgins, on the other hand, may cycle between the Golgi and the ER. There are three type-II transmembrane golgins in animal cells, namely giantin, golgin-84, and CASP (CCAAT-displacement proteins alternatively spliced product) [28, 36, 55, 56]. A fourth golgin, golgin-67, has been proposed to be a transmembrane protein [56], but there are good reasons for thinking that this is not the case since the Golgi targeting sequence does not fulfill the criteria expected for a true transmembrane domain [15]. The transmembrane domains of giantin, golgin-84, and CASP show a high degree of sequence similarity. In particular, key tyrosine and histidine residues are completely conserved. When the conserved tyrosine residue is mutated to leucine, CASP no longer localizes to the Golgi and accumulates in the ER instead [36]. Whether the mutant protein fails to exit the ER or is not retained in the Golgi is unknown. If the latter case is true, it could mean that wild type CASP cycles between the ER and Golgi. This is supported by the observation that CASP redistributes to the ER upon BFA treatment [36].

Golgin-84 is also likely to cycle between these compartments since BFA treatment also causes its redistribution to the ER [34]. Strictly speaking, this means that golgin-84 and CASP are not part of the Golgi matrix according to the definition that matrix components remain separate from the ER upon BFA or dominant negative Sar1p treatment. Nevertheless, these two golgins are clearly important structural components of the Golgi and have several other features in common with bona fide matrix proteins.

Thus, it seems that all structural components of the Golgi recycle to either the ER or the cytoplasm. This leads to the question of the extent to which the Golgi apparatus can be thought of as an independent organelle with its own identity [9]. An analysis of the effects of BFA and dominant negative Sar1p suggests that the Golgi depends, at least in part, on the ER for its existence [7]. Indeed, confocal video microscopy studies of the yeast *Pichia pastoris* have demonstrated, at least in this organism, that the Golgi forms de novo at sites intimately linked with the transitional ER, where COPII-coated vesicles form to deliver material to the Golgi [57]. Golgins may play a key role in maintaining this link, since the depletion of *Drosophila melanogaster* p115 results in the disorganization of both the Golgi stack and transitional ER sites [58].

However, since some matrix components like GM130 and GRASP65 cannot be induced to relocalize to the ER by treatment with BFA and dominant negative Sar1p [8], the Golgi does have a separate identity from the ER as defined by these proteins. There are some caveats to this, as under conditions where ER exit is blocked by the drug H89 in the presence of BFA even Golgi matrix proteins appear to be redistributed to the ER [59]. Recent evidence suggests that it may only be under a restricted set of experimental conditions that components of the Golgi redistribute to the ER [60]. Pecot and Malhotra used a protein-trapping approach to demonstrate that, contrary to some theories of Golgi dynamics, ER-resident proteins and Golgi-resident enzymes do indeed colocalize upon BFA treatment but do not come into contact with one another during mitosis, suggesting that the Golgi remains a distinct organelle throughout the mammalian cell cycle [60].

Nevertheless, considerable changes do occur to Golgi structure during mitosis [1]. We will now briefly consider how these changes are regulated by kinases and how morphologically similar changes during apoptosis are regulated by caspases.

### 5. Changes in Golgi structure during mitosis and apoptosis

At the onset of mitosis in animal cells, the Golgi apparatus fragments into numerous tubulo-vesicular structures known as mitotic Golgi fragments (MGFs), which contain various structural components of the Golgi such as GM130 and GRASP65. During cytokinesis, the MGFs become partitioned between the two daughter cells and then coalesce to reform a functional, perinuclear Golgi with a typical ribbon-like appearance [61]. These large-scale changes in Golgi structure are accompanied by changes in the phosphorylation states of many resident Golgi proteins through the activities of many mitotically regulated kinases and phosphatases. A full review of these events is beyond the scope of this article; for a more detailed review see Preisinger and Barr (2001) [62]. Nevertheless, a few examples will serve to illustrate the principles by which Golgi structure is regulated during mitosis.

The paradigm for the regulation of the Golgi structure by phosphorylation is the interaction between p115 and GM130, which has been reported to contribute to COPI- and COPII-coated vesicle docking and cisternal stacking [20, 21, 23, 31]. This interaction is disrupted at the onset of mitosis by the phosphorylation of GM130 on serine25 by Cdk1 [63]. This is thought to be of significance for the mitotic breakdown of the Golgi apparatus due to the continued budding of transport vesicles while p115–
The onset of mitosis [66,67]. GRASP55, on the other hand, thus may contribute to the unstacking of Golgi cisternae at GRASP65 appears to alter its ability to self-oligomerise, and thus may contribute to the unstacking of Golgi cisternae at the onset of mitosis [66,67]. GRASP55, on the other hand, has been shown to be an in vitro substrate for the mitogen-activated protein (MAP) kinase ERK2, which possibly acts downstream of MEK1 [68]. This fits with reports that MEK1 (and its upstream activator Raf1) is required for the fragmentation of the Golgi during prophase [69–71]. The relative importance of some of these phosphorylations is disputed however [63,72] and a full picture of the events leading to the mitotic breakdown of the Golgi is still to emerge.

The Golgi also fragments during apoptosis although the proteolytic cleavage of Golgi structural proteins, rather than phosphorylation, is the key regulatory mechanism. Again, the reader is referred to a more detailed review for a fuller discussion of this topic [73].

The reason for the apoptotic breakdown of the Golgi is unclear but it may be necessary in order to prevent unregulated protein transport to the cell surface during cell death. Indeed, the cleavage of giantin and the SNARE protein syntaxin5 by caspase-3 is accompanied by a block in ER to Golgi transport [74].

Golgin-160 is cleaved by caspase-2 and GRASP65 is a target of caspase-3 during apoptosis [75,76]. The expression of cleavage-resistant forms of these proteins delays apoptotic Golgi fragmentation, demonstrating the importance of these proteins for Golgi structure. Another golgin, p115, is a target for caspases 3 and 8 and, again, Golgi fragmentation was slowed by the expression of cleavage resistant p115 [77]. Interestingly, the C-terminal cleavage product of p115 appears to translocate into the nucleus and have a pro-apoptotic effect, indicating a signalling function for the Golgi during apoptosis [77].

Thus, large-scale alterations in Golgi morphology during mitosis and apoptosis are regulated by the actions, respectively, of kinases and caspases on Golgi structural components. Under normal, interphase conditions however, small GTPases are more important for the dynamic regulation of Golgi structure and function.

6. Regulation by small GTPases

The Rab family of small, Ras-related GTPases consists of over 60 members in the human genome [78]. They have been shown to be involved in almost all stages of vesicle transport, from vesicle budding and motility to tethering and fusion [79]. They accomplish all of these through the nucleotide-dependent recruitment of a variety of effector proteins.

Different members of the Rab family are able to target to specific membrane compartments within the cell, and several Rab proteins localize to specific sub-domains of the Golgi. Precisely how this targeting is achieved is unclear but, once a Rab protein is localized and activated, it is able to recruit its effectors either from the cytosol or from within the two-dimensional plane of the membrane. These effectors can then be organized and a series of protein–protein and protein–lipid interactions set up to establish a specific membrane sub-domain. At the Golgi, these effectors include numerous members of the golgin family [14].

Thus, through their ability to cycle between the cytoplasm and membranes, Rab proteins play a key role in specifying the identities of particular membrane domains [80,81]. The constant flux of protein and lipid through the Golgi in both anterograde and retrograde directions would lead to a rapid mixing of all the sub-domains of the Golgi if Rab proteins were unable to dynamically regulate the golgins and allow the assembly and disassembly of functional membrane domains. The participation of Rab proteins at all stages of vesicle transport, from cargo selection to docking and fusion, suggests that vesicles too may be looked on as Rab-organized membrane sub-domains, which, during their lifetime, are able to physically separate from one membrane and join with another.

Some golgins are specifically recruited to the Golgi by their interaction with their cognate Rab protein. For example, Rab6 recruits both Bicaudal-D (and its associated dynactin subunits) as well as the golgin TMF to Golgi membranes [37,38,82]. Similarly, p115 is recruited to membranes by Rab1 [83], although its interaction with GM130 probably also plays a role in its membrane association at the cis-Golgi [26]. GM130 itself relies on its interaction with GRASP65 for its localization to Golgi membranes [27]. Nevertheless, GM130 interacts with three different Golgi-localized Rab proteins, Rabs 1, 2, and 33b [50,84–86]. Likewise, the GRASP55-binding golgin, golgin-45, is a Rab2 effector [50], while the integral membrane golgin, golgin-84, interacts with Rab1 [34,35]. The function of the interaction between Rabs and golgins already associated with Golgi membranes may be to organize the golgins within the plane of the membrane or Rab binding may induce conformational changes in the golgin resulting in alterations in the golgin activity. Rab binding might similarly alter the affinity of a tethering factor for its binding partner. On the other hand, larger Rab-induced conformational changes in a golgin might directly assist vesicle docking and fusion by ‘collapsing’ a tethered vesicle onto its target membrane. Breaks in the coiled-coil sequence of a golgin might act as hinges to facilitate such a process.

Rab proteins are not the only small GTPases known to regulate components of the Golgi matrix. The ARL family
of ARF-related GTPases, of which there are ten members in humans, seems to have diverse functions in vivo. However, at present only two members of the family, ARL1 and ARL3/ARFRP1, are known to be important for Golgi structure and function. Other ARLs may function elsewhere in the cell in processes with little to do with membrane traffic, since ARL2 is found to complex with both a phosphodiesterase and the tubulin-folding cofactor D [87,88].

Mammalian ARL1 was shown to be important for Golgi structure since the overexpression of a constitutively active mutant (Arl1Q71L) resulted in large, unstacked Golgi cisternae while a dominant negative mutant (Arl1T31N) caused the Golgi to disappear [89]. ARL1 was then shown to bind the GRIP domain of golgin-245 [90]. The GRIP domain is an ~45 amino acid motif found in at least four mammalian golgins (p230/golgin-245, golgin-97, GCC88, and GCC185) and one yeast golgin (Imh1p), and targets these proteins to the trans-Golgi/TGN. The mutation of an invariant tyrosine residue in the GRIP domain abolishes the interaction with ARL1 [90]. Studies in budding yeast demonstrated that Arl1p was required for the Golgi localization of Imh1p and that the GRIP domain of Imh1p mediated this interaction [94,95]. In addition, Arl1p was also required for the localization of exogenously expressed mammalian GRIP domains to the yeast Golgi [95]. The latter result suggests that ARL1 recruits several GRIP domain-containing golgins to the trans-Golgi and that this mechanism is conserved throughout evolution.

The crystal structure of ARL1 bound to the GRIP domain of golgin-245 has recently been solved, providing an intriguing insight into the mechanism by which this complex is targeted to the Golgi [96,97]. The GRIP domain of golgin-245 forms a homodimer, with each monomer separately binding an ARL1-GTP molecule. The critical tyrosine residue of the GRIP domain protrudes into a “selectivity pocket” in ARL1 consisting of the switch regions of the protein. When ARL1 is bound to GDP, the switch regions are in a different conformation and the “selectivity pocket” is closed [96,97]. The ARL1–GRIP complex is thought to associate with membranes by orientating itself such that the N-terminal myristoyl groups of ARL1 and tryptophan residues near the C-termini of the GRIP domains can all insert into the lipid bilayer. The simultaneous binding of two ARL1 molecules by the GRIP domain dimer is likely to stabilize the association of the golgin with the membrane [96,97].

But how does ARL1 specifically target to the Golgi? Yeast Arl3p (ARFRP1 in humans) is required for the association of Arl1p and Imh1p with the Golgi although it doesn’t interact directly with either of these proteins [94,95]. Arl3p is also not myristoylated at its N-terminus. Rather, the membrane association of Arl3p is dependent upon it being acetylated at its N-terminus by the NatC complex [98,99]. The Golgi integral membrane protein Sys1p (Sys1 in humans) is also required for Arl3p localization, possibly acting as a receptor for acetylated Arl3p. Thus, Sys1p localizes Arl3p to the Golgi, resulting in the recruitment of Arl1p and Imh1p [98,99]. How Sys1p is correctly localized, and how Arl3p mediates the recruitment of Arl1p are outstanding questions. Nevertheless, this GTPase cascade demonstrates the exquisite regulation involved in localizing golgins such as the GRIP proteins and maintaining correct Golgi structure and function. It is also reminiscent of the GTPase cascade leading to the recruitment of the Rab-GTPase Sec4p on to Golgi to plasma membrane targeted vesicles in budding yeast [100]. In this case, two GTPases Ypt31p and Ypt32p, recruit Sec2p, the exchange factor for Sec4p, to the vesicles and this promotes GDP–GTP exchange on Sec4p and thus its membrane association [100].

Whether other GTPases interact with golgins in a similar way to the ARL–GRIP domain system remains to be seen. An indication that this may be a common theme comes from the recent finding that the golgin GMAP-210 and its yeast homologue Rud3p contain a GRIP-related ARF-binding (GRAB) domain [101]. Like the GRIP domain the GRAB domain is positioned toward the C-terminus, and binds to a small GTPase of the ARF/ARL family, in the case of Rud3p this is Arl1p [101]. However, this is insufficient for Golgi-targeting and Erv14p, a small recycling transmembrane protein of the ER-Golgi cargo receptor family is also needed for Golgi localization of Rud3p.

7. The role of the cytoskeleton in maintaining Golgi structure

So far, we have only considered the role of Golgi matrix proteins and their regulatory molecules in organizing the Golgi apparatus. But additional factors are also required to maintain normal Golgi structure, including microtubule-based motor proteins and other microtubule binding proteins.

The mammalian Golgi apparatus depends on an intact microtubule network to maintain its normal morphology. If microtubules are disrupted by, for example, treatment with nocodazole, the Golgi fragments into “mini-stacks” which, although scattered throughout the cytosol, are still capable of mediating membrane traffic [102]. Repolymerisation of microtubules allows the mini-stacks to move to the microtubule organizing centre (MTOC) of the cell where they coalesce to reform the Golgi ribbon [103,104].

Maintenance of an intact Golgi at the MTOC appears to require a balance of plus- and minus-end directed motor protein activity. The minus-end directed motor protein cytoplasmic dynein 1 localizes to the Golgi and the inhibition of its function by the overexpression of the dynactin subunit p50 results in a dispersal of the Golgi similar to that seen upon nocodazole treatment [105,106]. A second isoform of cytoplasmic dynein also localizes to the
Golgi and plays a role in maintaining its organization [107]. Dynein requires the multi-subunit protein dynactin to target it to specific cargo and stimulate its motor activity [108]. As discussed above, Rab6 and the golgin Bicaudal-D are important for the specific recruitment of dynactin and dynein to Golgi membranes [37,38], and a model for this process is presented in Fig. 1d.

Plus-end directed motors of the kinesin family are also found at the Golgi. Conventional kinesin is Golgi localized and its inhibition by either anti-sense application or antibody microinjection causes the Golgi to collapse into a compact structure at the MTOC [109–111]. Thus, a continuous “tug of war” between the plus- and minus-end directed microtubule-based motors determines the position and contributes to the organization of the Golgi apparatus [112]. In addition to Bicaudal-D, other proteins are likely to be involved in linking Golgi membranes to microtubules and motor proteins. Candidates include Hook3 at the cis-Golgi [113], and CLIP-59 at the trans-Golgi [114]. Another golgin that has been suggested to function in linking the Golgi to the cytoskeleton is GMAP-210 [115–117]. GMAP-210 may act as a recruitment factor for gamma-tubulin, and thus promote the nucleation of microtubules that help to organize the Golgi ribbon around the centrosome [116]. However, these findings are somewhat controversial since the gamma-tubulin binding and centrosome targeting portion of GMAP-210 identified by Rios and coworkers [116] maps to the same region as the Golgi-targeting ARF1-binding GRAB domain discussed previously [101]. This matter is discussed in more detail elsewhere [118].

The actin cytoskeleton is also important for maintaining Golgi structure in mammalian cells. Treatment of cells with cytochalasin D to disrupt actin filaments caused the Golgi to adopt a more clustered morphology instead of the usual extended ribbon [119]. An extensive actin network is necessary for normal Golgi structure. The main player is the Golgi matrix, a complex of coiled-coil golgins, and their regulatory GTPases associated motor proteins cooperate to dynamically regulate the Golgi apparatus. The maintenance of cell polarity (reviewed in [134]). Interactions between the cytoskeletal networks and components of the Golgi matrix are likely to be of key importance in this and other processes.

8. Conclusions and future perspectives

Numerous different proteins are involved in organizing the Golgi apparatus into its typical, stacked, ribbon-like structure. The main player is the Golgi matrix, a complex network of proteins such as the GRASPs, the expanding family of coiled-coil golgins, and their regulatory GTPases of the ARF, ARL, and Rab families. The dictionary definition of the word ‘matrix’ is “...a surrounding substance within which something else originates, develops, or is contained” and this fits well with the role of the Golgi matrix.

Many golgins act as membrane tethers although many of the coiled-coil proteins labelled as such may, in fact, be much more active in their function than previously thought. Golgin-84, for instance, has been proposed to have a specific role in the lateral organization of Golgi stacks [34], while p115 may actively promote SNARE complex assembly for membrane fusion [22]. One can imagine other possible functions for coiled-coil ‘tethering proteins’ such as negative regulation of membrane fusion by keeping membranes apart and providing transient binding sites for vesicles in order to limit their diffusion. The stacking of Golgi cisternae, for example, would represent a case in which membranes are tethered together but do not undergo fusion. Additional components of the matrix surely await discovery, and the recent demonstration that the cis-Golgi golgin GM130 activates two kinases involved in cell migration and polarity hints at hitherto unexpected roles.
for these proteins [135]. What is clear is that no single golgin alone can be said to be responsible for maintaining Golgi structure. Rather, it is the complex network of interactions between these proteins that is important.

As the Golgi apparatus is a highly dynamic organelle, the regulation of the matrix is essential for it to fulfil its function. Small GTPases of the Rab and ARL families appear to carry out this regulatory role, and an appreciation of how these GTPases themselves are controlled will be a key goal of future research. The identification of specific GEFs and GAPs will help our understanding of how GTPases and their effector proteins are organized in time and space to actively maintain both Golgi structure and function. A number of important studies have begun to define the structural basis of how a small set of these factors act [136–138], but much more work is needed to identify the full set of regulators acting on the nearly 60 human Rab GTPases. Larger changes in Golgi morphology during mitosis and apoptosis are regulated by protein kinases and caspases respectively. Again, only a few proteins have been examined in any detail, so for the most part the exact molecular details of the changes seen in mitosis and apoptosis remain to be elucidated.

Finally, various cytoskeletal networks also play a role in organizing the Golgi. Microtubules and associated motor proteins appear to be of particular importance. An understanding of how the cytoskeleton interacts with components of the Golgi matrix will undoubtedly help us in the future to form a complete picture of how the Golgi apparatus is held together.

Acknowledgements

We thank the reviewers for making a number of important suggestions and the members of the Barr group for reading the manuscript. The Max-Planck Society supports research in the laboratory of FAB.

References


394

B. Short et al. / Biochimica et Biophysica Acta 1744 (2005) 383–395


