Biogenesis of the plant Golgi apparatus

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Abstract

It has long been assumed that the individual cisternal stacks that comprise the plant Golgi apparatus multiply by some kind of fission process. However, more recently, it has been demonstrated that the Golgi apparatus can be experimentally disassembled and the reformation process from the ER (endoplasmic reticulum) monitored sequentially using confocal fluorescence and electron microscopy. Some other evidence suggests that Golgi stacks may arise de novo in cells. In the present paper, we review some of the more recent findings on plant Golgi stack biogenesis and propose a new model for their growth de novo from ER exit sites.

Introduction

It is often stated that the major difference in the organization of the animal and plant Golgi apparatus is that, in the former, the Golgi stacks predominantly form a perinuclear ribbon around the nucleus and, in the latter, the Golgi apparatus takes the form of numerous individual cisternal stacks which appear to be randomly scattered throughout the cytosol [1,2]. However, this is not strictly true as in many animal systems such as Drosophila and in some mammalian cells, the Golgi apparatus can also exist as many discrete units in the cells [3]. The key features that define the plant Golgi apparatus are the extensive mobility of the individual stacks in vacuolated cells owing to their interaction with the actin/myosin cytoskeleton, the lack of an intermediate compartment between the ERES (endoplasmic reticulum exit sites) and the cis-Golgi [4–7], and that Golgi bodies, unlike their animal counterparts, stay intact throughout the cell division process. It has been suggested that this latter fact is due to the requirement of the cell, during the second half of mitosis, to be active in the secretion of new membrane and wall material to the phragmoplast which supports the developing cell plate [2]. One consequence of this is that Golgi stacks need to be partitioned between the two daughter cells during mitosis, which poses the questions as to when in the cell cycle does the number of Golgi stacks double and are the number of Golgi stacks in the cell related to the volume of cytoplasm or the secretory activity of the cell?

There are surprisingly few reports on the process of Golgi stack replication and its relationship to cell division in plants. It has been suggested previously that Golgi duplication takes place during mitosis or during cytokinesis [8,9]. However, one of the most careful studies on Golgi duplication was carried out in rapidly frozen shoot apical meristem cells of Arabidopsis, where it was shown by a stereological and electron tomographic analysis that the interphase complement of approx. 35 Golgi stacks doubled during G2-phase immediately before mitosis [10]. These authors, however, did not demonstrate the mechanisms by which the number of Golgi stacks duplicated. It has also been shown in various algae, including the desmid Closterium ehrenbergii, that the number of Golgi bodies duplicates just prior to mitosis (presumably in G2-phase) and the author assumed this was by division of pre-existing stacks [11].

Therefore the exact mechanism of Golgi stack biogenesis is far from clear. It has traditionally been assumed that pre-existing Golgi stacks simply divide by fission. This could be in a cis–trans direction or in a trans–cis direction. It is even possible that stacks could cleave into two along the medial cisternae. It has recently been suggested from another electron tomographic study that this fission process can be attributed to the formation of two distinct cis-cisternae on a pre-existing stack [12]. Following the cisternal maturation model of Golgi transport, these two cisternae would act as templates for new cis-cisternae and, over time, two separate trans-cisternae would mature and the stack be cleaved in two [12]. More correctly, this should be called duplication by maturation. A final option is that Golgi stacks arise de novo from the ER (endoplasmic reticulum) [13], a concept that challenges the notion of the Golgi apparatus as an independent organelle, a status which has recently generated much debate in the animal and yeast Golgi community [14].

Although Golgi bodies can exist as structural entities within the cytoplasm separate from the ER in meristematic cells [13,15], in many cell types it is accepted that Golgi bodies and the ER coexist as some kind of structural entity linked by the protein complement of the ERES [16–18]. Such a tight association has recently been demonstrated by laser trapping and manipulation of individual Golgi stacks, which resulted
in apparent growth (stretching) and remodelling of attached ER tubules [19]. It is therefore possible that the biogenesis of individual Golgi stacks and regulation of their number is controlled by the number and activity of the ERES on the ER. This concept is considered in more detail in the rest of the present paper and finally we present a tentative model of Golgi biogenesis from the ER.

Deconstruction and reconstruction of the Golgi

It has long been known that the macrocyclic fungal metabolite BFA (brefeldin A) will completely block secretion in most eukaryotic cells. In plants, its action is certainly slower than in mammalian cells and yeasts, but in many tissues, it does result in the redistribution of Golgi membrane markers into the ER [4,20,21]. Some groups have termed the resulting organelle in plants an ER–Golgi hybrid [22]. Whether such a treatment results in the complete disassembly of all of the components of the Golgi stack is not yet known, and there has been considerable debate in the mammalian literature as to whether a protein scaffold composed of Golgi matrix proteins exists as an independent entity after induced disassembly of Golgi cisternae [14,23,24]. Such a redistribution of Golgi membrane markers into the ER can also be mimicked by inhibition of cargo exit from ERES through disruption of the COPII (coatamer protein II) coat complex by the expression of a GTP-locked version of the small Sar1p GTPase. Expression of such a construct is lethal to plants, but can be achieved through transient expression [17,25,26] or more controllably when expression is driven by an inducible promoter [27]. Remarkably, recovery from BFA-induced Golgi disassembly can be achieved simply by washing out the drug and incubating tissues in medium or water [20,28]. This can even take place when both actin filaments and microtubules are depolymerized and when protein synthesis is inhibited by cycloheximide [20]. This early work on Golgi live-cell imaging was carried out using marker constructs for the Golgi such as ST (signal anchor sequence of a rat sialytransferase)–GFP (green fluorescent protein) or a soya bean mannosidase 1–GFP construct [5]. More recently, however, a wider range of plant Golgi proteins, including matrix proteins [29], a number of N-glycan-processing enzymes [30,31], nucleotide–sugar transporters [32] and SNAREs (soluble N-ethylmaleimide-sensitive fusion protein-attachment protein receptors) [33] have been cloned, which has permitted analysis using fluorescent protein constructs [27] (J. Schoberer, J. Runions, H. Steinkellner, R. Strasser, C. Hawes and A. Osterrieder, unpublished work). Figure 1 shows the redistribution of GFP–AtCASP with respect to the medial/trans-Golgi membrane marker ST–mRFP (monomeric red fluorescent protein) during BFA treatment [At (Arabidopsis thaliana) CCAAT-displacement protein alternatively spliced product], AtGolgin-84 or Atp115 are likely candidates to take over important tethering functions at the plant Golgi stack [15,29].

When secretion in tobacco leaf epidermal cells is blocked with BFA or by inducing the expression of the GTP-locked Sar1p, the sequential fate of both a range of processing enzymes and matrix proteins (coiled/coil golgins) can be followed using fluorescent protein constructs [27] (J. Schoberer, J. Runions, H. Steinkellner, R. Strasser, C. Hawes and A. Osterrieder, unpublished work). Figure 1 shows the redistribution of GFP–AtCASP with respect to the medial/trans-Golgi membrane marker ST–mRFP (monomeric red fluorescent protein) during BFA treatment. Whereas ST–mRFP fluorescence is fully redistributed to the ER, the cis-Golgi matrix protein continues to label punctate structures which co-locate with the ERES marker Sar1-GTP–YFP (yellow fluorescent protein) [27]. Extending the analysis using fluorescent cis- and trans-Golgi markers, the first event on depletion of secretion in leaves appears to be the loss of trans-Golgi enzymes and golgins such as
the GRIP-domain-containing protein AtGRIP [J. Schoben, J. Runions, H. Steinkellner, R. Strasser, C. Hawes and A. Osterrieder, unpublished work). This is followed by a sequential redistribution of medial and cis-Golgi enzymes into the ER. cis-Golgi matrix proteins are relocated to the ER or cytoplasm and additionally continue to label puncta that presumably represent ERES. Reformation of Golgi stacks appears to be in the opposite sequence with cis-matrix proteins gathering at the ERES followed by cis- and medial enzymes. Finally, trans-enzyme location is preceded by the trans-matrix proteins being brought back from the cytosol to Golgi membranes, an event which may be mediated by small GTPases of the Rab and Arl families [35,36]. We do not yet know the role of matrix proteins in Golgi biogenesis. For instance, are the golgins at the cis-face required to construct a three-dimensional scaffold at the ERES to permit the assembly of a cis-cisterna and do they tether the Golgi to the ERES or can a cisternal stack generate de novo from ER membranes without the requirement for accessory proteins? Likewise, are golgins needed to establish cis–trans Golgi stack polarity or to aid the differentiation of cisterneae?

The sequential redistribution of Golgi membrane proteins in a trans–cis sequence upon BFA treatment suggests the existence of a novel retrograde pathway between the Golgi apparatus and the ER. COPI (coatamer protein I) vesicles have been shown to be retrograde vectors in mammalian cells [37] and it is known that COPI-independent pathways also exist [38,39]. In the experiments described above, the same sequence of events was reported after Sar1p inactivation and after BFA treatment. The latter in most instances would result in the loss of any COPI carriers [21]. We can therefore conclude that, in plants, a COPI-independent retrograde pathway between the Golgi apparatus and the ER has to exist. There are several mechanical options open to speculation for the retrograde route, including direct tubular connections between the trans-Golgi and the ER, a retrograde network of tubules linking the periphery of cisternae or a direct flow of membrane backwards through the Golgi stack between cisternae. Golgi tubules and connections to the ER have been reported in the plant literature for many years [1,40,41], but, to date, we have no evidence of their contribution, if any, towards cargo and membrane transport in either direction. It is, however, interesting to note that the concept of tubules or direct connections between Golgi cisternae is gaining support in the mammalian Golgi community, offering alternative transport models to the vesicle trafficking and cisternal maturation theories [42,43].

Electron microscopy of Golgi stack formation

Surprisingly, very few ultrastructural studies have been carried out on Golgi stack reformation after BFA treatment. A study on BY-2 cells after BFA washout showed budding profiles on the ER surface and that the first event in stack reformation was vesicle clustering to form a tubulo-vesicular network [28]. These clusters differentiated into what were termed ‘mini-Golgi’ stacks of up to 250 nm in diameter. Unfortunately, no COPII coat components could be localized to the regenerating Golgi stacks or ER during the study, but COPI coat components ARF (ADP-ribosylation factor) and γ-COP (coatamer protein γ) were located to the tubulo-vesicular clusters very early on in stack regeneration, indicating that there may be very early retrograde sorting events occurring within the regenerating stack. We have carried out an electron tomographic study of the mini-Golgi stacks in these BY-2 cells. After high-pressure freezing and freeze-substitution of cells recovering from BFA treatment, it is clear both from individual tomographic slices (Figure 2A) and three-dimensional reconstructions (Figures 2B and 2C) that at this early stage in stack formation cisternae are interconnected by membranous connections between their hubs which reduce in frequency as the stacks mature. Thus, the potential exists at this stage of Golgi stack development for a free flow of cargo, be it membrane-bound or soluble, between individual cisternae as has been proposed to occur in mammalian Golgi [42,43].

Figure 2  Tomographic analysis of reforming Golgi stacks after BFA treatment (10 μg·ml⁻¹) of BY-2 cells

Material was rapidly frozen in a Bal-Tec HPM 010 high-pressure freezer and freeze-substituted in osmium acetone. Single tilt series from 250 nm thick sections (±55°) were collected with a JEOL 2010 transmission electron microscope at 200 kV and tomograms were made with ITOMO software. Reconstructions were made with IMOD (University of Colorado, Boulder, CO, U.S.A.). (A) Single section from a tomogram of a mini-Golgi stack 15 min after BFA washout. Note the large vesicles at the forming trans-face and connections between cisternae. Scale bar, 100 nm. (B) Reconstruction of the mini-Golgi stack in (A). Connections between the differently coloured cisternae and the trans-vesicles can be seen (arrows). Scale bar, 100 nm. (C) Reconstruction of a reforming Golgi stack 60 min after BFA washout. The cisternae have matured, but still show connections between their hubs (arrows). Scale bar, 100 nm.
An interesting feature of stack regeneration was that the mini-Golgi stacks appeared with time to overgrow to nearly double their mature diameter. Between 120 and 150 min after BFA removal, these large stacks cleaved into two by cis-trans fission, although some anomalous bipolar dividing stacks were also reported [28]. A very similar sequence of events during Golgi regeneration has also been reported in the unicellular alga *Chlamydomonas* *noctigama* [44]. It is interesting to note that such a sequence of events for Golgi stack enlargement and cis-trans fission was proposed to explain Golgi stack division in hyphe of the filamentous oomycete *Pythium* which is closely related to the algae [41]. This process of enlarging the Golgi stack before division appears to be different to that described for *Arabidopsis* root meristem Golgi bodies, which may rely on the differentiation of two separate cis-cisternae on a pre-existing stack [12]. Alternatively, if individual stack biogenesis is contingent on the activity of the ERESs, one could envisage a scenario where ERES fission could result in the formation of two separate cis-cisternae linked to a single medial cisterna (see Figure 3).

**De novo biogenesis of Golgi stacks**

Although we can experimentally dismantle the cisternal stacks making up the plant Golgi apparatus and reconstruct the organelle, is there evidence to suggest that these processes occur in Nature? Certainly, imaging of the yeast *Pichia pastoris* has clearly shown in vivo biogenesis of the small stacks from ERES scattered over the ER [45]. As it has been shown that Golgi stacks are often intimately associated with ERES [17], it could be speculated that the number of Golgi stacks in a cell is regulated by the number of ERES on the ER surface. Thus a prelude to the de novo formation of a new Golgi stack would be the formation of a new ERES. It has been shown that visualization of the ERES using fluorescent protein constructs can vary depending on the nature of the construct and the nature of the cargo passing through the ERES. The fluorescence of the small GTPase Sar1p, tagged with YFP, at the ERES in tobacco leaf epidermal cells was greatly enhanced if it was co-expressed with a Golgi membrane marker such as ERD2–GFP, but not with a secretary marker such as sec–GFP [17]. Recruitment of the COPII coat protein Sec24 was also enhanced on the co-expression of ERD2–GFP or by the expression of a chimaeric Golgi-targeted marker protein with a diacidic ER export signal [46]. More importantly, there was a significant increase in the number of ERESs with the expression of the Golgi-targeted constructs, but not with sec–GFP [46]. The conclusion from this work was that the plant ER can respond to a sudden demand in signal-mediated ER export by both the recruitment of COPII components to existing ERESs and the de novo formation of new ERESs. Nonetheless, it was assumed that, with a bulk flow secretory product such as sec–GFP, the existing ERES capacity was sufficient to accommodate the extra protein flow through the system. As in this study, the ERES markers always co-located with the ERD2 Golgi marker, it must be concluded that the increase in secretory activity also resulted in a concomitant increase in the number of Golgi stacks in the cell.

If, as the above results suggest, Golgi stacks can be formed de novo in response to changes in targeted cargo expression, can this event be visualized in vivo? One approach is to utilize photoconvertible fluorescent proteins spliced to Golgi-targeted constructs. Proteins such as Kaede [47] or Dendra [48] can be photoconverted from green fluorescence into red on brief irradiation with UV or violet light. Thus, in theory, a population of Golgi stacks can be converted from green into red fluorescence. On incubation of the tissue, any new Golgi marker protein would emit in the green and, if transported to existing Golgi stacks, would give a mixed red and green (yellow) signal, whereas new Golgi stacks should fluoresce green. In a study on tobacco BY-2 cells, ST–Kaede-labelled Golgi were photoconverted from green into red and incubated for up to 30 h (B. Satiat-Jeunemaître and S. Brown, personal communication). In this time period, the majority of Golgi stacks turned from red to yellow upon the delivery of newly synthesized ST–Kaede to the photoconverted Golgi, but a small green-only population of stacks was recorded. This green-only population can be interpreted as de novo formation of new Golgi stacks or, a less likely event, as loss of red fluorescence from a small population of existing stacks. Clearly, this approach needs to be validated with a range of photoconvertible proteins combined with a range of Golgi markers in different tissues before any firm conclusions can be drawn as to the prevalence of de novo Golgi formation in higher plants.

**A model for Golgi stack biogenesis**

From the literature and data reported above, we propose a tentative model for the de novo biogenesis of the plant Golgi stack from the ER (Figure 3).

(i) An ER exit site is generated on the surface of the ER membrane. This may be orchestrated by the recruitment of Sec16p, which has been suggested to be the key protein for organizing and defining the ERES [49,50]. Two isoforms of Sec16p are encoded by the *Arabidopsis* genome, but are yet to be cloned and localized [18,49]. The exit site is then defined by a full complement of proteins including SNAREs, tethering factors such as TRAPP (transport protein particle) and COG (conserved oligomeric Golgi) complexes [34], golgins including Atp115 [15,29], AtGolgin-84 and AtCASP [27,29] homologues, the Sec12p guanine-nucleotide-exchange factor [17], Sar1p GTPase and COPII coat components (Figure 3A, see [18] for a list). Such ERESs would be motile on the ER surface, but it is not yet known whether force generation is by myosins acting directly on the ER membrane (i.e. ERESs move with the surface of the ER [19,51]) or on the ERES themselves.

(ii) A COPII bud forms at the exit site and develops into a tubulo-vesicular network tethered to the ER possibly by cis-Golgi matrix proteins (Figures 3B and 3C). Whether free COPII vesicles are involved in this process in higher plants is not clear, although they have been reported in *Arabidopsis*
Figure 3 | Possible sequence of events in the de novo biogenesis of a Golgi stack from a motile ERES

(A) Differentiation of ERES (depicted as a brown oval in the ER) requires a defined set of proteins. (B) The first step in cisternal formation is the development of a coated bud (presumably COPII) at the exit site. (C) Development of a tubulo-vesicular network associated with the exit site. (D) Formation of a mini-Golgi stack and establishment of stack polarity aided by matrix proteins followed by migration of cis-located glycan-processing enzymes. (E) Localization of medial and trans-processing enzymes in the developing stack. (F) Overgrowth and fission of the new Golgi stack. (G) Alternative model for duplication of an oversize Golgi stack through division of the ERES and maturation of two new stacks. Figures are not drawn to scale.

Meristems [15]. COPI proteins may be involved in stack differentiation at this time point [28]. This process may be regulated by the Rab1 homologue RabD2A [52].

(iii) Fusion of the membranes of the tubulo-vesicular network results in the formation of a mini-Golgi stack (Figure 3D). Differentiation into cis- and trans-faces may be regulated by the recruitment of trans-located matrix proteins such as the TMF (TATA modulatory factor) homologue and the GRIP-domain-containing protein [35] (J. Schoberer, J. Runions, H. Steinkellner, R. Strasser, C. Hawes and A. Osterrieder, unpublished work). Cis-located transferases migrate into the stack followed by medial and trans-enzymes (Figures 3D and 3E). Movement of these type II transmembrane proteins would be via direct connections between cisternae.

(iv) New cisternae continue to mature at the cis-face and the Golgi stack continues to expand beyond its natural mature size and divides into two by cis–trans fission [28] (Figure 3F). Membrane connections between the cisternal hubs concomitantly reduce in number.

(v) Alternatively, the exit site could divide in two, giving rise to two new cis-cisternae that develop on the maturing medial cisternae and through maturation would eventually generate two new stacks of standard diameter (Figure 3G).

Conclusions

The model proposed in the present paper draws mainly on data derived from the study of the Golgi apparatus in leaf epidermal cells and suspension culture cells. Whether it...
holds true for other cell types, especially meristematic cells where Golgi can be free from as well as associated with the ER, remains to be determined. If so, then it would be expected that the Golgi–ER connection can be severed in much the same way as it is now accepted that the trans-Golgi network can probably form at and be released from the trans-face of the Golgi [7,12,13]. Future elucidation of this mechanism not only will undoubtedly rely on refinement of live cell imaging and application of high-resolution light microscope techniques, but also will require a careful time-resolved electron microscopy immunocytochemical study of protein location in developing Golgi stacks.

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