Golgi and Mitosis

Guest lecture by
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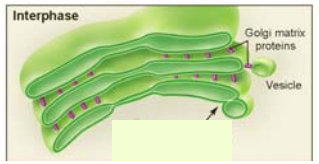
April 5, 2005

The Golgi apparatus performs three major functions

- operates as a carbohydrate factory for processing proteins and lipids moving through the secretory pathway
- serves as a station for protein sorting and transport from ER to PM and intracellular sites
- acts as a membrane scaffold onto which diverse signaling, sorting and cytoskeleton proteins adhere

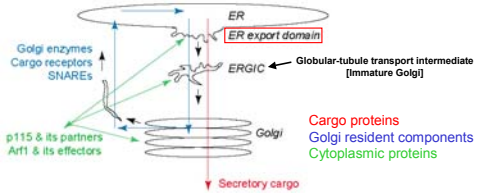
GOLGI

- Stacked array of cisternae and connecting tubules/vesicles
- Enormous diversity of proteins (> 1000 different types)
- Dynamically transform in response to cellular stimuli
  - eg: Mitosis, osmotic shock
    - proteins: transiently associate with the Golgi
      part of a large complex



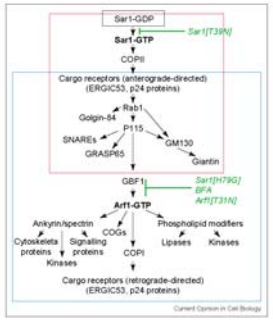
No class of Golgi proteins are stably associated with the Golgi (GFP imaging studies)

- Integral membrane proteins are continuously exiting and reentering the Golgi from ER ~60 min
  - Processing enzymes: Mannosidase II, Glactosyltransferase
  - SNAREs
  - secretory cargo receptors: ERGIC53, p24, KDEL (~10 min)
- Peripheral membrane associated proteins constantly exchange between membrane and cytosolic pools ~1 min
  - Arf1 and its effectors (Phosphotidylinositol kinases, lipases, signaling kinases)
  - coatomer, p115, GRASP
- Newly synthesized cargo proteins (integral and luminal) going to other places in the cell ~30 min
  - integral membrane and luminal proteins



Trafficking itineraries of proteins associated with the Golgi apparatus. All proteins that associate with the Golgi do so only transiently before moving to other destinations in the cell. Integral membrane and luminal proteins enter the Golgi via transport intermediates formed from ER export sites. Among those are cargo proteins (red) that pass through the Golgi to the cell surface or other cellular destinations and Golgi resident components (blue)(including Golgi enzymes, cargo receptors and SNAREs specific to the ER-Golgi system) which return to the ER through retrograde transport intermediates. Cytoplasmic proteins associated with the Golgi (green) (including Arf1 and its effectors, p115 and its binding partners) continuously bind to and dissociate from ER export domains, ERGIC or Golgi membranes.

The Golgi appears to undergo continuous outgrowth from and reconsumption by the ER through the formation of anterograde and retrograde transport intermediates.

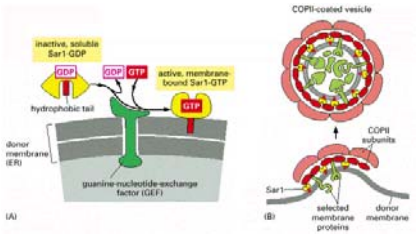


Schematic organization of Golgi biogenesis. **(a)** Recruitment of proteins involved in the biogenesis of the Golgi as a steady-state system is based on the sequential GTPase activities of Sar1 and Arf1. Note that different sets of interacting protein pathways associate with different compartments: ER (black box), ER export domain (red box), ERGIC and Golgi membranes (blue box).

ER
ER export domain
ERGIC and Golgi membranes

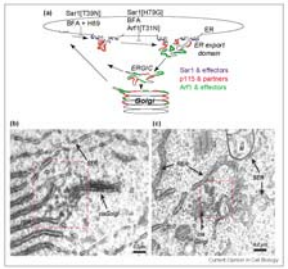
Sar1[T39N]:
No GTP binding
Sar1[H79G]:
Binds GTP but poor hydrolysis
Arf1[T31N]:
does not associate with membranes

SNAREs: Budding from ER export domain/fusion
GRASP 65: Sorting of p24 cargo receptors
GM130/Giantin: Tethering reactions between membrane and cytoskeleton
GBF1: exchange factor for Arf1 GTPase



A current model of COPII-coated vesicle formation.
(A) **The Sar1 protein is a coat recruitment GTPase.** Inactive, soluble Sar1-GDP binds to a GEF (called Sec12) in the ER membrane, causing the Sar1 to release its GDP and bind GTP. A GTP-triggered conformational change in Sar1 exposes its hydrophobic tail, which inserts into the ER membrane.
(B) Membrane-bound, active Sar1-GTP recruits COPII subunits to the membrane. This causes the membrane to form a bud, which includes selected membrane proteins. A subsequent membrane-fusion event pinches off and releases the coated vesicle. Other coated vesicles are thought to form in a similar way. In contrast to Sar1, the coat recruitment GTPase ARF contains a covalently attached fatty acid chain that functions similarly to the hydrophobic tail of Sar1 as a regulated membrane anchor; it is retracted in the GDP-bound state and exposed in the GTP-bound state.

Differentiation and functioning of ER export domains are important and is prior to formation of ERGIC



p115 and its interacting partners (GM130, giantin, GRASP65, SNAREs, Rab1 GTPase, GBF1) have to be fully active for ER export domains (*activated by Sar1*) to give rise to ERGIC

ER to Golgi transition. **(a)** Effects of mutations in Sar1 and Arf1, as well as the drugs BFA and H89 on assembly of ER export domains. **(b,c)** Ultra-structure of endoplasmic reticulum and Golgi apparatus in COS-7 cells. Note the fenestration of the cis-Golgi in (c) and the continuity of membranes between the smooth ER and the Golgi in both (b) and (c) within these cells. The dashed red box indicates the complex organization of an ER export domain.

The multi-step process of Golgi biogenesis

• Sar1 GTPase activity initiates the process of Golgi biogenesis by COPII-mediated sorting of specific integral membrane proteins (ERGIC53, p24, KDELR)

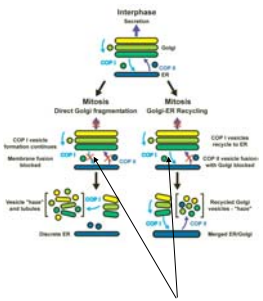
• Clustering of these proteins results in changes in bilayer thickness and composition at these sites leading to the recruitment and activation of molecules like Rab1 which in turn recruits p115 to these sites

• The ability of p115 to interact with SNAREs and matrix proteins then causes the nascent ER export sites to differentiate into ERGIC by stimulating membrane transformation and fusion events locally

• Transformation of ER export domains into "dynamic transport intermediates" by Arf1 and GBF1 and effector proteins (ankyrin, spectrin, COPI, signaling proteins, phospholipid-modifying enzymes)

When fused together these intermediates comprise the Golgi!

Two models of Golgi fragmentation during mitosis



Golgi fragmentation in mitosis.
During interphase, the Golgi receives secretory cargo from the ER via the COP II vesicle formation pathway. Depending on their nature, secretory cargo then travel through the Golgi apparatus by a combination of vesicular transport involving COP I vesicles and cisternal maturation. A retrograde COP I vesicle pathway connects Golgi cisternae and the ER to allow recycling of Golgi enzymes and integral membrane proteins of the transport machinery such as the SNAREs.

Two models have been proposed to explain the fate of the Golgi in mitosis.
The first is the **direct fragmentation model**, where Golgi cisternae are consumed by continued COP I vesicle budding in the absence of membrane fusion, which is blocked in mitosis. This generates a pool of tubules and vesicles (Golgi haze) containing Golgi enzymes and structural proteins.
The second is the **ER recycling model**, where forward transport into the Golgi by COP II vesicles is blocked, but COP I recycling back to the ER continues until Golgi proteins are redistributed to this compartment. Some Golgi proteins may then exit the ER via the COP II pathway, but these vesicles are unable to fuse with a Golgi compartment during mitosis, and remain in equilibrium with the ER.

There are several strategies for Golgi inheritance

de novo formation

fission

disassembly–reassembly

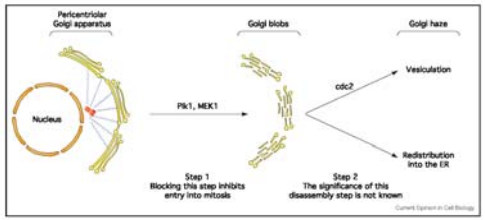
•Glick and coworkers examined the Golgi during mitosis in the budding **yeast** *Pichia pastoris* and found *de novo* formation of Golgi in a daughter cell.

• In the **protozoa** *Toxoplasma gondii*, Warren and coworkers reported binary fission of Golgi for its inheritance. In this case, the Golgi splits in half before mitosis and segregates into the two daughter cells.

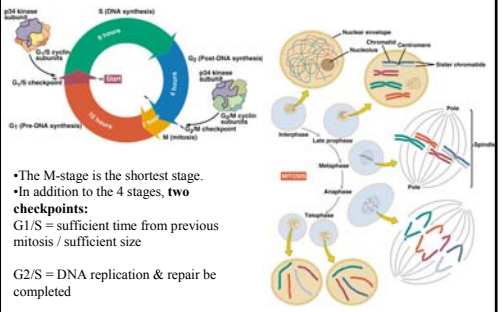
• In contrast, **animal cells** utilize the strategy of **disassembly–reassembly**.

During mitosis, the Golgi apparatus is fragmented into thousands of vesicles and short tubules that are dispersed throughout the cytoplasm. Some or all of them might be absorbed into ER. This is a matter of contention.

At telophase, the Golgi apparatus is rapidly reassembled from the fragments within each daughter cell.
This disassembly–reassembly process is regulated by phosphorylation.



The pericentriolar stacks of Golgi cisternae undergo two sequential fragmentation reactions during mitosis in mammalian cells. Plk1 and MEK1 are required for converting these stacks into tubulo-reticular elements (Golgi blobs). These Golgi blobs undergo further disassembly. The extent to which Golgi blobs are processed in this second disassembly reaction is controversial. The current extreme proposals are conversion into small vesicles by a process that requires cdc2 kinase, or fusion with the ER. It has been reported recently that preventing the fragmentation of the pericentriolar Golgi stacks into Golgi blobs inhibits entry of mammalian cells into mitosis. It would be most valuable to ascertain the effect of blocking the second disassembly step on the fate of the Golgi membranes *in vivo* and on the progression of cells through mitosis.

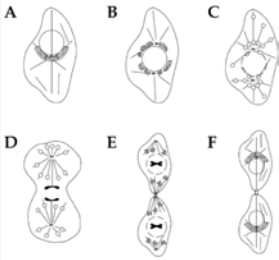


- The M-stage is the shortest stage.
- In addition to the 4 stages, **two checkpoints:**

G1/S = sufficient time from previous mitosis / sufficient size

G2/S = DNA replication & repair be completed

Model for Golgi apparatus disassembly and partitioning during mitosis



(***A***) In most cells, the Golgi apparatus exhibits a polarized position in **interphase**, at one side of the nucleus and next to the centrosome.

(***B***) At the **G2/M transition**, the Golgi ribbon reorganizes and adopts a more perinuclear localization. This reorganization may be coordinated by the separation of centrosomes, and the association of Golgi membranes with the two microtubules organizing arrays.

(***C***) Fragmentation into Golgi stacks continues throughout **prophase**, and coincident with nuclear envelope break down, Golgi stacks rapidly fragment to yield mitotic Golgi clusters that are repositioned around the spindle poles and by astral microtubules.

(***D***) As cell division ensues, Golgi clusters associate with microtubules, forming ring-like structures that are partitioned along with each spindle pole and one complement of sister chromatids.

(***E***) At the end of **cytokinesis**, reformed Golgi stacks are positioned both close to the **midbody** and to the daughter cell centrosome, then slowly converge to reform the Golgi ribbon as shown in (***F***)

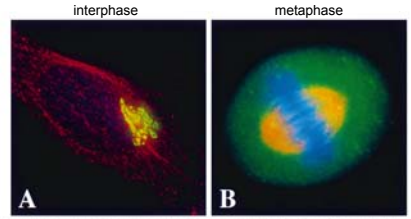


FIG. 1. Mitotic Golgi disassembly. Golgi (green), microtubule (red), and chromatin (blue) staining is shown for interphase (A) and metaphase (B) HeLa cells. The juxta nuclear interphase Golgi ribbon is composed of laterally connected stacked cisternal membranes. The dispersed hazy and punctate staining of the mitotic Golgi is composed of abundant small vesicles as well as larger vesicle clusters and cisternal remnants. This is visualized as a 2D projection from a deconvolved 3D data set using summation of pixel values in each plane. The Golgi marker is GPP130.

During M phase some steps are apparently blocked

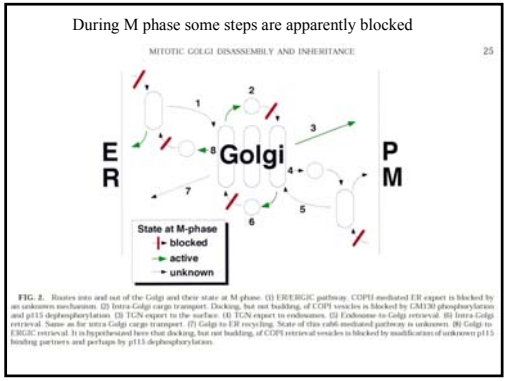


FIG. 2. Routes into and out of the Golgi and their state at M-phase. (1) ER/ERGIC pathway. COPII-mediated ER export is blocked by an unknown mechanism. (2) Intra-Golgi cargo transport. Docking, but not budding, of COPI vesicles is blocked by GM130 phosphorylation and p115 dephosphorylation. (3) TGN export to the surface. (4) TGN export to endosomes. (5) Endosome-to-Golgi retrieval. (6) Intra-Golgi retrieval. Same as for intra Golgi cargo transport. (7) Golgi to ER recycling. State of this rab6-mediated pathway is unknown. (8) Golgi to ERGIC retrieval. It is hypothesized here that docking, but not budding, of COPI retrieval vesicles is blocked by modification of unknown p115 binding partners and perhaps by p115 dephosphorylation.

Golgi membranes are first converted into small elements (**Golgi blobs**), which, in a cell type-specific manner, are further processed and appear diffusely dispersed (**Golgi haze**)

But what is the molecular description of this haze?

Does it represent Golgi membranes in the form of small vesicles or the relocation of Golgi enzymes into the ER?

Why Mammalian Cells Fragment the Golgi Apparatus So Extensively during Mitosis?

If the pericentriolar Golgi membranes are not fragmented into large blobs (GRASP65), cells remain arrested in G2 and do not enter mitosis.

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Golgi Membranes Remain Segregated from the Endoplasmic Reticulum during Mitosis in Mammalian Cells

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Summary

What happens to organelles during mitosis, and how they are apportioned to each of the daughter cells, is not completely clear. We have devised a procedure to address whether Golgi membranes fuse with the Endoplasmic Reticulum (ER) during mitosis via the detection of interactions between ER and Golgi proteins. This procedure involves coexpressing an FKBP-tagged Golgi enzyme with an ER-retained protein fused to FRAP in COS cells. Since treatment with rapamycin induces a tight association between FKBP and FRAP, one would expect rapamycin to trap the FKBP-fused Golgi protein in the ER if it ever visits the ER during mitosis. However, after the doubly transfected cells progress through mitosis in the presence of rapamycin, we find the Golgi protein in the newly formed Golgi stacks and not in the ER. Based on these results, we conclude that Golgi membranes remain separate from the ER during mitosis in mammalian cells.

ASSAY

•This assay relies on the ability of two proteins to conditionally bind in the presence of ligand.

•**Rapamycin**, a small molecule, binds to the FK506 binding protein (FKBP)

•The FKBP-rapamycin associated protein (**FRAP**) binds to the FKBP-rapamycin complex only in the presence of rapamycin.

•Fused FKBP to **Sialyltransferase**, an enzyme of the medial to trans Golgi cisternae, (ST, ST-FKBP), and FRAP to an ER retained version of the human invariant γ_0 chain protein (Ii, Ii-FRAP -HA tagged).

•If Golgi membranes were to be redistributed into the ER, Ii-FRAP will quickly and efficiently **trap ST-FKBP in the presence of rapamycin**.

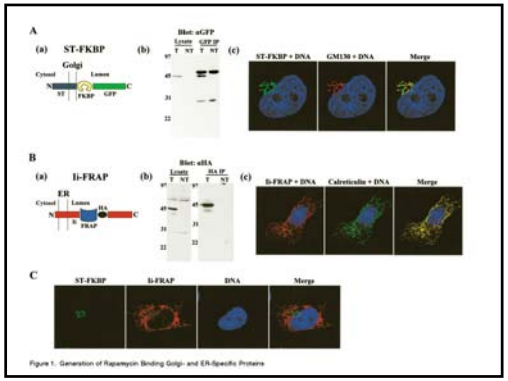


Figure 1. Generation of Rapamycin Binding Golgi- and ER-Specific Proteins

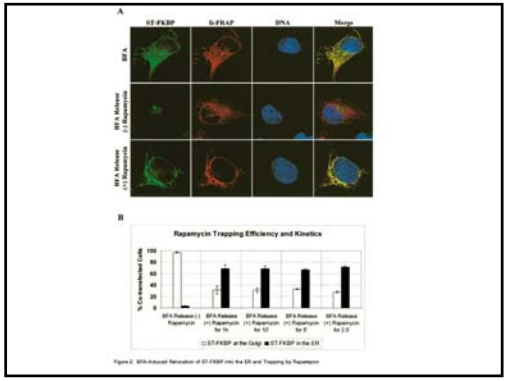


Figure 2. BFA-Induced Relocation of ST-FKBP into the ER and Trapping by Rapamycin

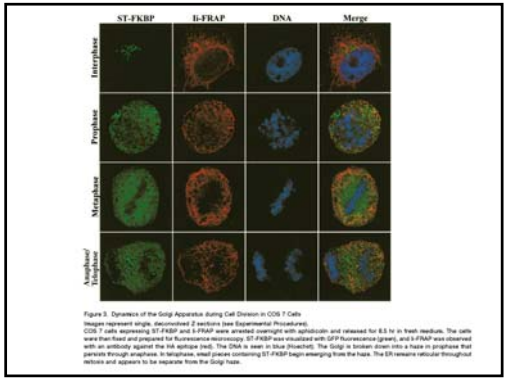


Figure 3. Dynamics of the Golgi Apparatus during Cell Division in COS 7 Cells

Images represent single, deconvolved Z sections (see Experimental Procedures). COS 7 cells expressing ST-FKBP and Ii-FRAP were arrested overnight with aphidicolin and released for 8.5 hr in fresh medium. The cells were then fixed and prepared for fluorescence microscopy. ST-FKBP was visualized with GFP fluorescence (green), and Ii-FRAP was observed with an antibody against the HA epitope (red). The DNA is seen in blue (Hoechst). The Golgi is broken down into a haze in prophase that persists through anaphase. In telophase, small pieces containing ST-FKBP begin emerging from the haze. The ER remains reticular throughout mitosis and appears to be separate from the Golgi haze.

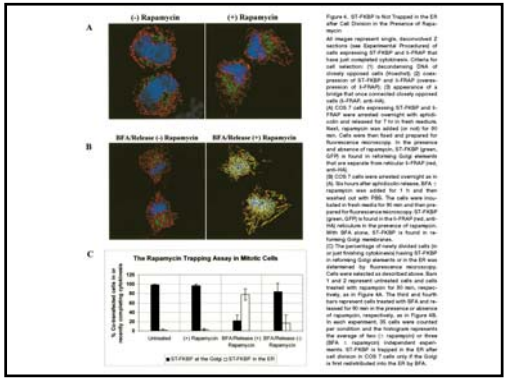


Figure 4. ST-FKBP is Not Trapped in the ER after Cell Division in the Presence of Rapamycin

All images represent single, deconvolved Z sections (see Experimental Procedures) of cells expressing ST-FKBP and Ii-FRAP that have just completed cytokinesis. Criteria for cell selection: (1) daughter cells, (2) closely opposed cells (Hoechst), (3) coexpression of ST-FKBP and Ii-FRAP (coexpression of Ii-FRAP), (4) appearance of a bridge that once connected closely opposed cells (Ii-FRAP, anti-HA).

(A) COS 7 cells expressing ST-FKBP and Ii-FRAP were arrested overnight with aphidicolin and released for 7 hr in fresh medium. Next, rapamycin was added (or not) for 90 min. Cells were then fixed and prepared for fluorescence microscopy. In the presence and absence of rapamycin, ST-FKBP (green, GFP) is found in reforming Golgi elements that are separate from reticular Ii-FRAP (red, anti-HA).

(B) COS 7 cells were arrested overnight as in (A). Six hours after aphidicolin release, BFA ± rapamycin was added for 1 h and then washed out with PBS. The cells were incubated in fresh media for 90 min and then prepared for fluorescence microscopy. ST-FKBP (green, GFP) is found in the Ii-FRAP (red, anti-HA) reticulum in the presence of rapamycin. With BFA alone, ST-FKBP is found in reforming Golgi membranes.

(C) The percentage of newly divided cells (in or just finishing cytokinesis) having ST-FKBP in reforming Golgi elements or in the ER was determined by fluorescence microscopy. Cells were selected as described above. Bars 1 and 2 represent untreated cells and cells treated with rapamycin for 90 min, respectively, as in Figure 4A. The third and fourth bars represent cells treated with BFA and released for 90 min in the presence or absence of rapamycin, respectively, as in Figure 4B. In each experiment, 35 cells were counted per condition and the histogram represents the average of two (± rapamycin) or three (BFA ± rapamycin) independent experiments. ST-FKBP is trapped in the ER after cell division in COS 7 cells only if the Golgi is first redistributed into the ER by BFA.

Conclusions

…… regarding the fate of Golgi membranes during mitotic progression

Mitotic Golgi fragments remain separate from the ER

We still don't know precisely whether biogenesis of the Golgi apparatus in daughter cells is from preexisting Golgi elements or occurs *de novo*.

Box 1 Golgi-associated signaling and regulatory molecules

Heterotrimeric G proteins [4]
Gi(3), Gs, G $\beta\gamma$

Small G proteins [4,36,42,43,53,73,75*]
Arf1, Arf3
Arf family members
Ras, Rac, Cdc42
Rab family members

Protein kinases [3,5,55,71,76,78,79]**
Phosphotidyl-inositol kinases
PKA
PKC isoforms
Casein kinase isoforms
Calmodulin kinase

Protein Kinase D

Myt1 kinase

Polo-like kinases, Plk- 3, Sak1
MEK kinases, MEKK2, MEKK4
Mammalian Ste20 kinases, YSK1 and MST4

Cytoskeletal regulatory proteins [3,4,57]
Dynein, dynamin
Myosin I, II, V, VI
Ankyrin isoforms

Spectrin isoforms
Kinesins
IQGAP, WASP/Arp2/3

Phospholipases [3,5,55,56]**
Phospholipase A_2

Phospholipase D_2

Others [3,4,5,77*,80]**
Cullin2, Cullin3
Endothelial nitric-oxide synthase (eNOS)
Phosphotidylinositol transfer protein, Nir2
Phosphatidylinositol phospholipid phosphatase, PTEN2
Tankyrase1, Tankyrase 2
CtBP/BARS

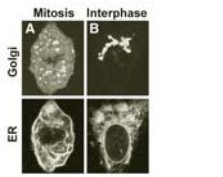
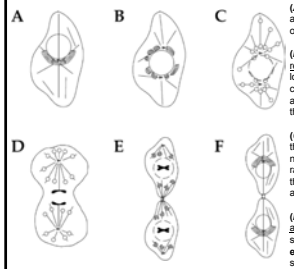


Figure 2. **Morphologically distinct ER and Golgi compartments exist in both interphase and mitotic cells.** The ER localized translocon component Sec61β and the Golgi marker *N*-acetylgalactosaminyl-transferase 2 were imaged in interphase and mitotic cells. At all time points the Golgi and ER remained morphologically distinct, despite significant differences in their morphology in mitotic compared with interphase cells. Images are courtesy of M. Axelsson, taken from Axelsson and Warren (2004).

Model for Golgi apparatus disassembly and partitioning during mitosis



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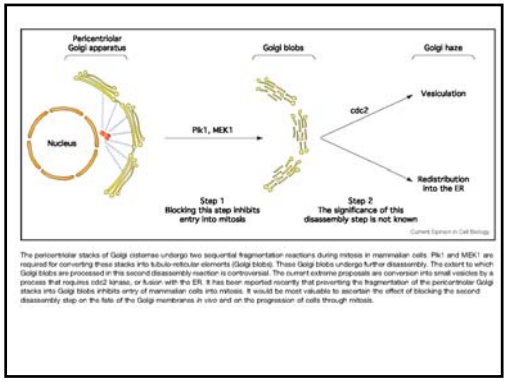

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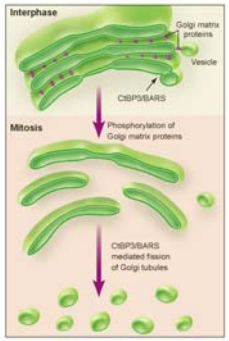
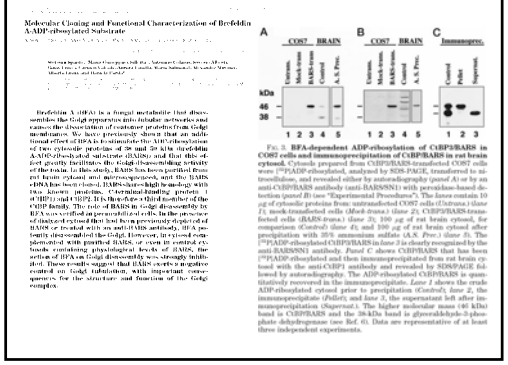


Splitting the difference. During interphase, the Golgi matrix proteins cross-link cisternae into stacks and tether transport vesicles to the rims of the cisternae. The CtBP3/BARS protein may contribute to the splitting off of transport vesicles from the cisternal rims. During cell division (mitosis), phosphorylation of the matrix proteins results in loss of stacking and vesicle tethering. The stacks convert to tubules that are accessible to CtBP3/BARS, which fragments them into numerous small vesicles. These accumulate alongside transport vesicles generated from the cisternal rims when tethering is inhibited. In the absence of CtBP3/BARS, the Golgi membranes cannot undergo fission and accumulate as tubules. Golgi membrane fission induced by CtBP3/BARS is required for proper entry of cells into mitosis.



Molecular Cloning and Functional Characterization of Brefeldin A-ADP-ribosylated Substrate

Brefeldin A (BFA) is a fungal metabolite that disassembles the Golgi apparatus into tubular networks and causes the dissociation of coatomer protein from Golgi membranes. We have previously shown that an additional effect of BFA is to stimulate the ADP-ribosylation of two cytosolic proteins of 38 and 50 kDa (brefeldin A-ADP-ribosylated substrate (BARS)) and that this effect greatly facilitates the Golgi-disassembling activity of the toxin. In this study, BARS has been purified from rat brain cytosol and microsequenced, and the BARS cDNA has been cloned. BARS shares high homology with two known proteins, C-terminal-binding protein 1 (CtBP1) and CtBP2. It is the 50-kDa ubiquitous member of the CtBP family. The role of BARS in Golgi disassembly by BFA was verified in permeabilized cells. In the presence of dialyzed cytosol that had been previously depleted of BARS or treated with an anti-BARS antibody, BFA potently disassembled the Golgi. However, in cytosol supplemented with purified BARS, or even in control extracts containing physiological levels of BARS, the action of BFA on Golgi disassembly was strongly inhibited. These results suggest that BARS exerts a positive control on Golgi tubulation, with important consequences for the structure and function of the Golgi complex.

A COS7 BRAIN — B COS7 BRAIN — C Immunoprec.
Untrans. / Mock-trans. / BARS-trans. / Control / A.S. Prec. / Control / Pellet / Superna.
kDa
46
38
1 2 3 4 5 — 1 2 3 4 5 — 1 2 3

FIG. 3. **BFA-dependent ADP-ribosylation of CtBP3/BARS in COS7 cells and immunoprecipitation of CtBP/BARS in rat brain cytosol.** Cytosols prepared from CtBP/BARS-transfected COS7 cells were [^{32}P]ADP-ribosylated, analyzed by SDS-PAGE, transferred to nitrocellulose, and revealed either by autoradiography (*panel A*) or by an anti-CtBP/BARS antibody (anti-BARS/SN1) with peroxidase-based detection (*panel B*) (see "Experimental Procedures"). The lanes contain 10 μg of cytosolic proteins from: untransfected COS7 cells (*Untrans.*) (*lane 1*), mock-transfected cells (*Mock-trans.*) (*lane 2*), CtBP3/BARS-transfected cells (*BARS-trans.*) (*lane 3*); 100 μg of rat brain cytosol, for comparison (*Control*) (*lane 4*); and 100 μg of rat brain cytosol after precipitation with 35% ammonium sulfate (*A.S. Prec.*) (*lane 5*). The [^{32}P]ADP-ribosylated CtBP3/BARS in *lane 3* is clearly recognized by the anti-BARS/SN1 antibody. *Panel C* shows CtBP/BARS that has been [^{32}P]ADP-ribosylated and then immunoprecipitated from rat brain cytosol with the anti-CtBP1 antibody and revealed by SDS/PAGE followed by autoradiography. The ADP-ribosylated CtBP/BARS is quantitatively recovered in the immunoprecipitate. *Lane 1* shows the crude ADP-ribosylated cytosol prior to precipitation (*Control*), *lane 2*, the immunoprecipitate (*Pellet*), and *lane 3*, the supernatant left after immunoprecipitation (*Supernat.*). The higher molecular mass (46 kDa) band is CtBP/BARS and the 38-kDa band is glyceraldehyde-3-phosphate dehydrogenase (see Ref. 6). Data are representative of at least three independent experiments.

CtBP/BARS induces fission of Golgi membranes by acylating lysophosphatidic acid

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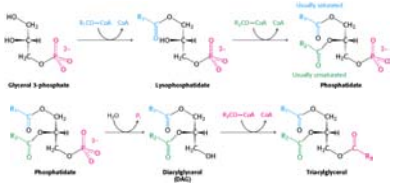
Membrane fission is essential in intracellular transport. Acyl-coenzyme As (acyl CoAs) are important in lipid remodelling and are required for fission of COPI-coated vesicles[1]. Here we show that CtBP/BARS, a protein that functions in the dynamics of Golgi tubules[2], is an essential component of the fission machinery operating at Golgi tubular networks, including Golgi compartments involved in protein transport and sorting. CtBP/BARS-induced fission was preceded by the formation of constricted sites in Golgi tubules, whose extreme curvature is likely to involve local changes in the membrane lipid composition. We find that CtBP/BARS uses acyl-CoA to selectively catalyse the acylation of lysophosphatidic acid to phosphatidic acid both in pure lipidic systems and in Golgi membranes, and that this reaction is essential for fission. Our results indicate a key role for lipid metabolic pathways in membrane fission.



- The first step in the synthesis of both phospholipids for membranes and triacylglycerols for energy storage is the synthesis of ***phosphatidate*** (diacylglycerol 3-phosphate).
- In mammalian cells, phosphatidate is synthesized in the endoplasmic reticulum and the outer mitochondrial membrane.
- It is formed by the addition of two fatty acids to *glycerol 3-phosphate*, which in turn is formed primarily by the reduction of dihydroxyacetone phosphate, a glycolytic intermediate, and to a lesser extent by the phosphorylation of glycerol.
- **Glycerol 3-phosphate is acylated by acyl CoA to form *lysophosphatidate*, which is again acylated by acyl CoA to yield phosphatidate.**
- These acylations are catalyzed by *glycerol phosphate acyltransferase*

Mitotic Golgi Partitioning Is Driven by the Membrane-Fissioning Protein CtBP3/BARS

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Organelle inheritance is an essential feature of all eukaryotic cells. As with other organelles, the Golgi complex partitions between daughter cells through the fission of its membranes into numerous tubulovesicular fragments. We found that the protein CtBP3/BARS (BARS) was responsible for driving the fission of Golgi membranes during mitosis in vivo. Moreover, by in vitro analysis, we identified two stages of this Golgi fragmentation process: disassembly of the Golgi stacks into a tubular network, and BARS-dependent fission of these tubules. Finally, this BARS-induced fission of Golgi membranes controlled the G_2-to-prophase transition of the cell cycle, and hence cell division.

C-terminus binding protein 3
Brefeldin A adenosine diphosphate ribosylated substrate

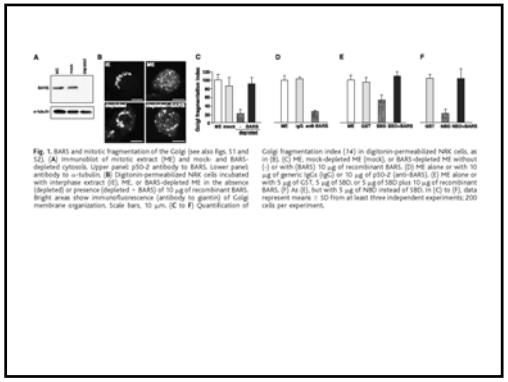


Fig. 1. BARS and mitotic fragmentation of the Golgi (see also figs. S1 and S2). (**A**) Immunoblot of mitotic extract (ME) and mock- and BARS-depleted cytosols. Upper panel: p50-2 antibody to BARS. Lower panel: antibody to α-tubulin. (**B**) Digitonin-permeabilized NRK cells incubated with interphase extract (IE), ME, or BARS-depleted ME in the absence (depleted) or presence (depleted + BARS) of 10 μg of recombinant BARS. Bright areas show immunofluorescence (antibody to giantin) of Golgi membrane organization. Scale bars, 10 μm. (**C** to **F**) Quantification of Golgi fragmentation index (*14*) in digitonin-permeabilized NRK cells, as in (B). (C) ME, mock-depleted ME (mock), or BARS-depleted ME without (-) or with (BARS) 10 μg of recombinant BARS. (D) ME alone or with 10 μg of generic IgG‡ (IgG) or 10 μg of p50-2 (anti-BARS). (E) ME alone or with 5 μg of GST, 5 μg of SBD, or 5 μg of SBD plus 10 μg of recombinant BARS. (F) As (E), but with 5 μg of NBD instead of SBD. In (C) to (F), data represent means ± SD from at least three independent experiments; 200 cells per experiment.

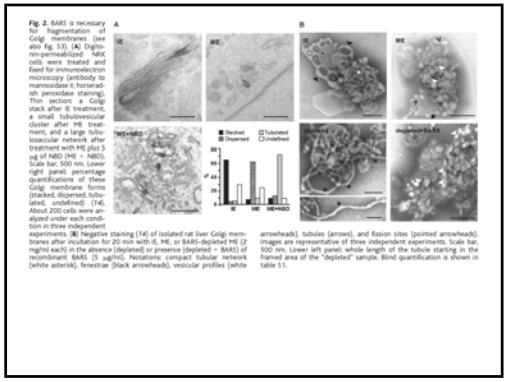


Fig. 2. BARS is necessary for fragmentation of Golgi membranes (see also Fig. S3). (**A**) Digitonin-permeabilized NRK cells were treated and fixed for immunoelectron microscopy (antibody to mannosidase II, horseradish peroxidase staining). Thin sections a Golgi stack after IE treatment, a small tubulovesicular cluster after ME treatment, and a large tubulosaccular network after treatment with ME plus 5 μg of NBD (ME + NBD). Scale bar, 500 nm. Lower right panel: percentage quantifications of these Golgi membrane forms (stacked, dispersed, tubulated, undefined) (*14*). About 200 cells were analyzed under each condition in three independent experiments. (**B**) Negative staining (*14*) of isolated rat liver Golgi membranes after incubation for 20 min with IE, ME, or BARS-depleted ME (2 mg/ml each) in the absence (depleted) or presence (depleted + BARS) of recombinant BARS (5 μg/ml). Notations: compact tubular network (white asterisk), fenestrae (black arrowheads), vesicular profiles (white arrowheads), tubules (arrows), and fission sites (pointed arrowheads). Images are representative of three independent experiments. Scale bar, 500 nm. Lower left panel: whole length of the tubule starting in the framed area of the "depleted" sample. Blind quantification is shown in table S1.

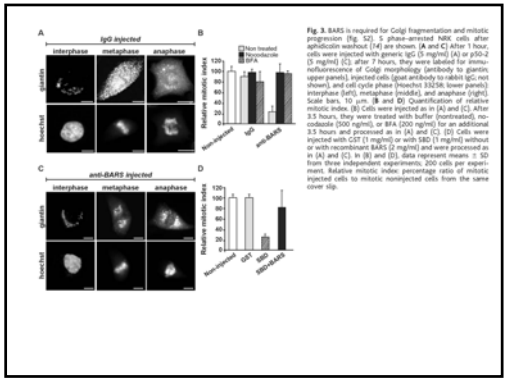


Fig. 3. BARS is required for Golgi fragmentation and mitotic progression (fig. S2). S phase–arrested NRK cells after aphidicolin washout (*14*) are shown. (**A** and **C**) After 1 hour, cells were injected with generic IgG (5 mg/ml) (A) or p50-2 (5 mg/ml) (C); after 7 hours, they were labeled for immunofluorescence of Golgi morphology (antibody to giantin; upper panels), injected cells (goat antibody to rabbit IgG; not shown), and cell cycle phase (Hoechst 33258; lower panels): interphase (left), metaphase (middle), and anaphase (right). Scale bars, 10 μm. (**B** and **D**) Quantification of relative mitotic index. (B) Cells were injected as in (A) and (C). After 3.5 hours, they were treated with buffer (nontreated), nocodazole (500 ng/ml), or BFA (200 ng/ml) for an additional 3.5 hours and processed as in (A) and (C). (D) Cells were injected with GST (1 mg/ml) or with SBD (1 mg/ml) without or with recombinant BARS (2 mg/ml) and were processed as in (A) and (C). In (B) and (D), data represent means ± SD from three independent experiments; 200 cells per experiment. Relative mitotic index: percentage ratio of mitotic injected cells to mitotic noninjected cells from the same cover slip.

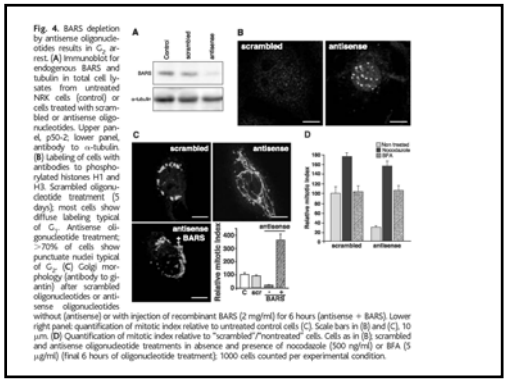
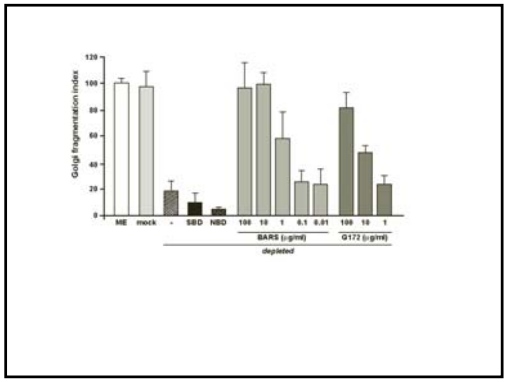
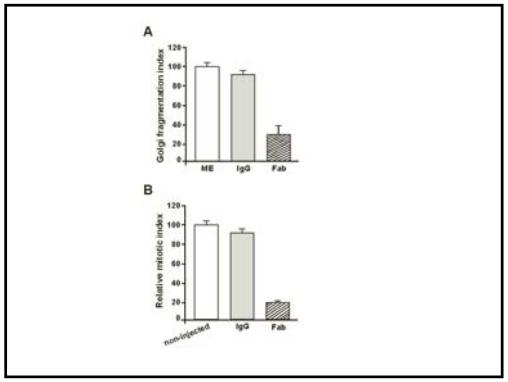


Fig. 4. BARS depletion by antisense oligonucleotides results in G_2 arrest. (**A**) Immunoblot for endogenous BARS and tubulin in total cell lysates from untreated NRK cells (control) or cells treated with scrambled or antisense oligonucleotides. Upper panel, p50-2; lower panel, antibody to α-tubulin. (**B**) Labeling of cells with antibodies to phosphorylated histones H1 and H3. Scrambled oligonucleotide treatment (5 days); most cells show diffuse labeling typical of G_1. Antisense oligonucleotide treatment; ~70% of cells show punctuate nuclei typical of G_2. (**C**) Golgi morphology (antibody to giantin) after scrambled oligonucleotides or antisense oligonucleotides without (antisense) or with injection of recombinant BARS (2 mg/ml) for 6 hours (antisense + BARS). Lower right panel: quantification of mitotic index relative to untreated control cells (C). Scale bars in (B) and (C), 10 μm. (**D**) Quantification of mitotic index relative to "scrambled"/"nontreated" cells. Cells as in (B); scrambled and antisense oligonucleotide treatments in absence and presence of nocodazole (500 ng/ml) or BFA (5 μg/ml) (final 6 hours of oligonucleotide treatment); 1000 cells counted per experimental condition.





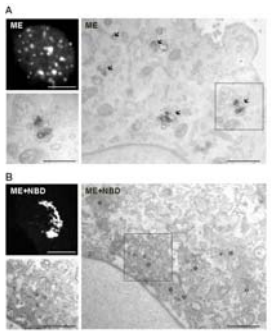


Table S1. BARS-dependent fragmentation activity of mitotic cytosol

	Preserved structures	Tubules	Vesicular profiles	Fission sites
Interphase cytosol	· · ·	-	-	-
Mitotic cytosol	-	·	· ·	· · ·
BARS-depleted mitotic cytosol	· ·	· · ·	-	-
BARS-depleted mitotic cytosol+recombinant BARS	-	-	· · ·	· · ·

The percent of structures characterized by the indicated phenotypes were scored as · · · (+75%), · · (+50%), · (+25%) or (+23%). The addition of BARS alone did not have any effect on Golgi structure. The data are from three independant experiments performed as described in Figure 2B. Fifty isolated Golgi structures (as shown in Figure 2B) were analyzed in a blind fashion for each sample.