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 (e.g., Mitosis, osmotic shock)
- 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, Glucosyltransferase
  - 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 (Phospholipid:inositol kinases, lipoases, 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

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

ER
- ER export domain
- ERGIC and Golgi membranes

SNAREs:
- Budding from ER export domain/fusion
- Sorting of p24 cargo receptors
- Tethering reactions between membrane and cytoskeleton
- Exchange factor for Arf1 GTPase (GBF1)

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

Globular-tubule transport intermediate (Immature Golgi)

Cargo proteins

Golgi resident components

Cytoplasmic proteins

Globular-tubule transport intermediate (Immature Golgi)

Cargo proteins

Golgi resident components

Cytoplasmic proteins
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 permits its interaction with other factors that insert the Sar1 coat into the ER membrane. 

(B) Membrane-bound, active Sar1-GTP recruits COPII subunits to the membrane. This causes the membrane to undergo the budding event, which results in the formation of a coated vesicle that pinches off into a newly formed ERGIC compartment. 

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, giant, GRASP65, SNAREs, Rab11 GTPase, GBF1) have to be fully active for ER export domains (activated by Sar1) to give rise to ERGIC.

The multi-step process of Golgi biogenesis

- Sar1 GTase activity initiates the process of Golgi biogenesis by COPII-mediated sorting of specific integral membrane proteins (ERGIC53, p24, KDEL).
- 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

- The direct fragmentation model, where Golgi cisternae are directly and independently fragmented by mitotic events (an event for example that has been reported in fission yeast). The Golgi fragments then reassemble upon completion of mitosis and the fragmented Golgi cisternae are reincorporated into the new ERGIC cisternae.

- The ER recycling model, where fragments are not incorporated into the new ERGIC cisternae but are instead recycled to the ER where they are then incorporated into new ERGIC cisternae. This model has been reported in mammalian cells and Drosophila melanogaster.

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 protozoan Toxoplasma gondii, Warren and coworkers reported binary fusion 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.

When fused together these intermediates comprise the Golgi!
• 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).

During M phase some steps are apparently blocked

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.
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 N 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.
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.
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 CEBP3/BARS

Cristina Hidalgo Carvajal, Matteo Baracchi, Stefania Spadis, Gabriele Barchirolli, Albino D’Ettorre, Daniela Corti

Organellar reassembly is an essential feature of all eukaryotic cells. As animal embryos develop, the Golgi complex, a network of flattened vesicles, residing in the perinuclear region, is fragmented into a series of smaller organelles. The Golgi complex is involved in the synthesis of membrane lipids, glycosylation of proteins, and sorting of proteins for secretion. The Golgi complex is composed of a series of stacked membranes that are continuous with the endoplasmic reticulum and the trans-Golgi network. The Golgi complex is involved in the synthesis of complex carbohydrates and lipid modifications, and it plays a crucial role in the sorting and transport of proteins and lipids from the endoplasmic reticulum to the plasma membrane.

This process is highly coordinated and requires the coordinated action of multiple proteins. One such protein is the Membrane-Fissioning Protein CEBP3/BARS (C-terminus binding protein 3/Brefeldin A diphosphate ribosylated substrate). This protein is involved in the regulation of Golgi membrane dynamics and organelle morphology. CEBP3/BARS is a member of the BARS family of proteins, which are involved in the regulation of membrane dynamics and organelle morphology.

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CEBP3/BARS is a protein that is involved in the regulation of Golgi membrane dynamics and organelle morphology. It is a member of the BARS family of proteins, which are involved in the regulation of membrane dynamics and organelle morphology.
Table 1: Real-time quantitative PCR analysis of transcripts/pred

<table>
<thead>
<tr>
<th>Treatment</th>
<th>100%</th>
<th>75%</th>
<th>50%</th>
<th>25%</th>
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<tbody>
<tr>
<td>Interferon</td>
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<tr>
<td>Mifepristone</td>
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<td>HU80-high volume</td>
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The percent of cultures demonstrated by the indicated phenotypes was tested at
- 100%: 1-3 PM a: -2.2% (p < 0.05). The inhibition of kinases activity
was assessed using the Student t-test. The data are presented as the mean ± standard
error of the mean (SEM) from at least three independent experiments. The data in
Figures 2A and 2B were analyzed with the Student t-test, for each sample.