ROLE OF THE CONSERVED OLIGOMERIC GOLGI COMPLEX (COG) AND ITS PARTNERS IN GLYCOSYLATION IN HUMAN CELLS.

Vladimir V. Lupashin
Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR, USA.
OUTLINE

• Introduction
  – Protein trafficking and glycosylation

• COG complex function
  – COG complex structure and localization
  – COG KD and KO cell lines
  – COG partners
  – mechanistic model of COG complex function
THE GLYCOSYLATION DYNAMICS AND THE GLYCOME

- Glycosylation is a template-independent, enzymatic process that adds one or more sugars to proteins and lipids.
- Adding the correct glycans to proteins or lipids employs at least 2% of the translated genome to generate thousands of molecular structures.
- The most important factors that determine glycosylation products: rate-limiting enzymes, their subcellular localization, the supply and localization of activated sugars.
- More than 100 distinct congenital disorders of glycosylation (CDG) are known.
- Subdivided into two types: CDG-I (glycan synthesis) and CDG-II (glycan addition to proteins/lipids).
VESICLE TETHERING, DOCKING AND FUSION PROTEIN MACHINERY

Modified from Bonifacino and Glick, 2004
OUTLINE

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• COG complex function
  – COG complex structure and localization
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  – COG partners
  – mechanistic model of COG complex function
• Conserved Oligomeric Golgi Complex. Proposed function of a vesicular tether for retrograde transport. Expressed in all Eukaryotes.

• Interacts with core components of vesicle trafficking including COPI coat proteins, Rab GTPases, SNAREs, and coiled-coil tethers

• Mutations in the COG complex lead to Congenital Disorders of Glycosylation (CDG II).
COG AND CONGENITAL DISORDERS OF GLYCOSYLATION TYPE II

- So what is the importance of proper Golgi homeostasis and Conserved Oligomeric Golgi complex?
- Glycosylation involvement: CDG type II
- Symptoms include; issues with clotting, elevated liver enzymes, mental and growth retardation, seizures, and-- in the most severe cases-- lethality within the first year of life

Renate Zeevaert et al., 2009
COG DEPLETION INDUCES ACCUMULATION OF VESICLES THAT CARRY GOLGI ENZYMES

**Diagram:***
- COG3 siRNA, days: 0, 3, 6, 9
- WB: Cog3p, Lamp2, CD44, GAPDH
- GlcNAcT1/GM130: Control, COG3 KD

**Images:**
- COG7 KD
- CTRL
CREATION OF HUMAN COG KNOCK-OUT CELL LINES

Transfection CRISPR Cas9 and gRNA plasmids

↓

Preliminary lectin stain

↓

Single cell sorting

↓

Growing up colonies

↓

Screening via lectin stain, western blot, and sequencing
HUMAN COG KNOCK-OUT CELL LINES

COG 1 KO  COG 2 KO  COG 3 KO  COG 4 KO  COG 5 KO  COG 6 KO

COG 7 KO  COG 8 KO

WT    KOs

WT

Jessica Bailey
HUMAN COG KNOCK-OUT CELL LINES

HEK Control

HEK COG 1 KO

HEK COG 6 KO

HEK COG 4 KO

HEK COG 6 KO

Irina Pokrovskaya & Jessica Bailey
CREATION OF HUMAN COG KNOCK-OUT CELL LINES

• COG KO phenotypic differences from the parental cell lines:
  – High mannose specific *Galanthus nivalis* lectin binding
  – Severely disrupted Golgi structure
  – Impaired trafficking
  – Accumulation of large vacuoles that appear to be of phagocytic/endocytic/lysosomal origin

• But still viable, able to freeze and thaw!
COG COMPLEX BI-LOBED MODEL

- Is this an over simplification of the model?
MEMBRANE-BOUND COG COMPLEX EXISTS IN SEVERAL COMBINATIONS

- COG subunits in HeLa membrane peaked in fraction corresponding to octameric as well as tetrameric subcomplexes

Rose Willett
While both proteins co-localize in the Golgi area, COG8 is mostly associated with 60-100 nm vesicle-like structures, while COG3 was mostly associated with larger 200-500 nm structures that likely represent rims of Golgi cisternae.
COG AND TMEM165 ARE NOT INTER-DEPENDENT

**WT**

<table>
<thead>
<tr>
<th>TMEM165</th>
<th>TMEM165 KO</th>
<th>COG3</th>
</tr>
</thead>
<tbody>
<tr>
<td>GalT</td>
<td>GalT</td>
<td>COG8</td>
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</table>

**WT**

<table>
<thead>
<tr>
<th>TMEM15</th>
<th>COG3 KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>GalT</td>
<td>GSII-647</td>
</tr>
</tbody>
</table>

*Leslie Climer*
MULTI EXPRESSION ASSAY

- All COG subunits tagged with same 3x Myc tag

Tetyana Kudlyk, Rose Willett
COG COMPLEX SELECTIVELY INTERACTS WITH PARTNERS

Willett et al., Cellular Logistics, 2014
LOBE B BINDS TO V-SNARE GS15 WHILE LOBE A INTERACTS WITH TETHER P115
WORKING MODEL
**Diagram:**

- **hCOG1**
- **TEV**
- **hCOG8**
  - **HA tag**
  - **TEV protease recognition site**

**Sequence:**

```
GSGTYPYDVPDYAGGGGGSGGGGGGGSTSGLETRDIRSENYLFQGDKDDDDKDGAQPYATGGGGGGGGGG...
```

**Images:**

**A:**
- GNL
- GNL-myc
- Merge

**B:**
- GNL
- COG1-TEV-COG8-Myc
- Merge

**C:**
- GNL

**D:**
- GNL PM labeling (Arb Units)

**Graph:**

- WT Rescue
- Hybrid Rescue
- COG8 Null

**Statistical Analysis:**

* Statistically significant difference p<.0001
CONCLUSIONS

• Vesicular trafficking is essential for protein glycosylation in human cells

• COG complex regulate Golgi vesicular trafficking

• All COG subunits are essential for its function

• COG lobe B sub-complex engages v-SNARE GS15 to tether Golgi vesicles via transient interaction with lobe A sub-complex
Lupashin Lab

Rose Willett  Tetyana Kudlyk  Irina Pokrovskaya

Jessica Bailey  Leslie Climer

Collaborators:
Maxim Dobretsov (UAMS)
Rainer Duden (University of Lubeck)
Victor Faundez (Emory University)
Hudson Freeze (Burnham Institute)
Fred Hughson (Princeton)
Fusun Kilic (UAMS)
Willy Morelle (University of Lille)
Uma Nagarajan (UNC)
James Paton (University of Adelaide)
Brian Storrie (UAMS)
Elizabeth Sztul (UBA)
Daniel Ungar (University of York)

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