Scientific Report

4th International Singapore Lipid Symposium
13-16 March 2012

Summary

The 4th ISLS, which has been held at National University of Singapore, has been an intriguing opportunity for both established and new scientists in the field lipid biology to acquire knowledge about the new perspectives, methodologies, and trends in the study of lipids. The Symposium was preceded by three workshops dealing with what turned to be the main focuses of the entire meeting: namely 1) lipid metabolism regulation, 2) mass spectrometry based lipidomics, 3) Large datasets curation. The symposium consisted of an intensive three-days programme with close to 40 oral contributions, discussion, and poster presentation sessions. Apart from the already mentioned leading topics, lipid imaging, lipid-protein interactions, membrane dynamics, and model systems lipidology were touched upon in several oral and poster presentations. Overall the symposium succeeded in covering different aspects of lipid-focused research bringing together diverse experiences and expertises and providing the participants with new ideas and perspectives on the topics in study.

Final Programme

Tuesday March 13

Workshop 1

Lipid metabolism and homeostasis-case studies

10:00-12:00

Song Baoling

Cholesterol metabolism: biosynthesis and adsorption

Scott Summers

Ceramides as modulators of cellular and whole-body metabolism

Workshop 2

Global standards for mass-spectrometry-based lipidomics

13:00 -15:00

Harald Kofeler

Development of a standardized shorthand lipid nomenclature

Dominik Schwudke

In which way should we address lipidomic data for bioinformatic analysis? Lessons from the Drosophila lipidome.

Guanghou Shui

The challenge of moving from comparative lipidomics to quantitative lipidomics

Todd Mitchell

Structural characterzation of molecular lipids: Where do we stand

Workshop 2

Curating lipidomic information

15:30-17:30

Ed Dennis

Curation challenges and solutions from the LIPID MAPS experience

Ionnais Xenarios (a PostDoc from the lab)

Biocuration from proteins to lipids

Andrej Shevchenko

Practical ways to visualize and rationalize lipidomics data

Wednesday March 14

9:20

Ed Dennis

Integration of genomics, proteomics, & metabolomics, finally! Lipid maps.

10:00

Rob Parton

High resolution localization of lipids: developments and applications

11:10

Xun Huang

Using Drosophila and C. elegans models to study lipid storage

11:50

Andreas Zumbusch

Coherent anti-stokes Raman scattering microscopy of intracellular dynamics

13:30

Peter Meikle

Plasma lipid profiling in type 2 diabetes and cardiovascular disease

14:10

Andrej Shevchenko

High resolution mass spectrometry for quantifying known and discovering new lipid molecules

14:50

Johanne Yew

Analysis of lipid pheromones from insects using mass spectrometry

16:00

Yuki Nakamura

Lipid metabolic switching to alter lipid levels in algae and plants

16:20

Brendan Prideaux

MALDI-MSI and LESA-MS for localization and identification of lipids in biological tissue

16:40

Christian Eggeling

Studying lipid-protein interactions in plasma membrane of living cells with super resolution STED microscopy

17:00

Guillaume Thibault

The unfolded protein response buffers the lethal effects of lipid disequilibrium

Thursday March 15

9:00

Patricia Bassereau

What can we learn on lipid trafficking with model membrane systems?

9:40

Herman Overkleeft

Chemical biology of glycolipid metabolism in relation to lysosomal storage disorders

10:50

Toon de Kroon

The yeast acyltransferase Sct1p regulates fatty acid desaturation by competing with the desaturase Ole1p

11:30

Anne-Claude Gavin

High-throughput approaches to lipid protein interactions

12:10

Markus Wenk

SLING, the Singapore Lipidomics Incubator

12:50

Todd Mitchell

xxBalls, brains, LESA™ and other 5500™ adventures

14:00

Takao Shimizu

Target lipidomics reveal critical molecules involved in disease onset and progression

14:40

Gabrilele Kastermuller

Genome metabolome interactions in large human populations

15:20

Benhur Lee

Mechanistic basis of broad-spectrum antivirals that target the physiochemical properties of viral lipid membranes to prevent virus-cell fusion and entry

16:30

Chakravarty Marella

BLAST inspired global analysis tool box for lipidomes

16:50

Adam Orlowski

Role of membrane cholesterol in hydrophobic matching and the resulting redistribution of proteins and lipids

17:10

Ajay Mahalka

Protein-phospholipid interactions: from biophysics to therapeutics

17:30

Lok Hang Mak

A small molecule mimicking a phosphatidylinositol (4,5)-bisphosphate binding pleckstrin homology domain

Friday March 16

9:00

Xu Chenqui

The regulation of immunoreceptor activation by phospholipid

9:40

Chung Shu Sin

Characterization of the two-protein complex that establishes lipid asymmetry at the outer membrane of Gram-negative bacteria

10:50

Igor Butovich

Qualitative and quantitative lipidomic assessment of human meibomian gland secretion

11:30

John Harwood

Algal lipids: topical and important

12:10

Ivo Feussner

The alphabet of galactolipids in Arabidopsis thaliana

14:00

Chye Mee-Len

Acyl-CoA-binding proteins from Arabidopsis and rice

14:40

Neil Clarke

Lipid metabolism and its gene regulation in model and non-model algae

15:20

Chew Fook Tim

Genetic strategies for oil yield improvement in oil crops and lipid productivity in algae

16:30

Giovanni D'Angelo

Molecular mechanism of metabolic branching in the synthesis of glycosphingolipids

16:50

Mathieu Blanc

25-hydroxycholesterol is directly coupled to the interferon response and has antiviral activities

17:10

Guan Xueli

Lipidomics of host-pathogen interactions: human macrophage as a cellular system to study functional implications of lipid metabolism during infection

17:30

Thusitha Rupasinghe

LCMS lipid profiling of intra-erythrocyte stages and intracellular organelles of Plasmodium falciparum

Description of the scientific content of the event

The scientific contributions to the 4^{th} ISLS can be grouped into six different thematic areas:

1) Lipidomics studies in vertebrates
2) Lipid imaging
3) Lipidomics and functional studies in non vertebrate organisms
4) Functional studies
5) Membrane dynamics studies
6) Protein-lipid interaction studies.

Lipidomics studies in vertebrates

This group of contributions included studies on different aspects of lipidome and lipidome remodelling in vertebrate organisms. The integration of different "omics" (i.e. genomics, transcriptomics, proteomics, and metabolomics) was extensively used by researchers included in this group to achieve either unbiased new biological hypothesis, or descriptive maps of lipid metabolism in both physiological and pathological conditions. In this group I also include contributions on technical advances in lipidome analysis.

Prof. Edward Dennis first introduced this specific topic in the workshop 3 by giving a lecture on the LIPID MAPS initiative, and then as a first speaker of the actual Symposium. Prof. Dennis described the effort undertaken by the scientists participating to the LIPID MAPS initiative in obtaining the most complete catalogue of lipid species structure and inferred biological function publicly available to date. In this frame and as an example Prof. Dennis reported the LIPID MAPS and his own lab experience in dissecting the lipidome remodelling consequent to endotoxin stimulation of macrophages as a model of inflammation and infection.

On a similar line Prof. Meikle described a study where lipidomic profiling was used to measure up to 300 different lipid species from plasma of either healthy, type 2 diabetic, and cardio-vascular disease affected human individuals. By this approach

Prof. Meikle reported a significant association of a number of new lipid species with metabolic syndrome and diabetes, whose systematic measure can be used both to gain new insights in the pathologies and as a new approach to risk stratification.
Prof. Andrej Shevchenko discussed in workshop 3 the possibilities to express changes in lipidome by considering different features of lipid molecules ad in the symposium about the advantages provided by rapid shotgun lipidomics in assessing global lipidomic responses to general environmental or genetic challenges.
Prof. Markus Wenk reported on SLING (the Singapore Lipidomics Incubator) as a coordinated effort among different academic and industrial entities to achieve a new working space producing new technologies and intellectual capital to be applied to diverse projects.
Prof. Takao Shimizu reported on his lipidomic approach to characterize the metabolic effects of cytosolic PLA2 deficiency in mice.
Dr Chakravarty Marella described an approach to compare lipid structures by the use of a dedicated alignment tool.
Prof. Igor Butovich reported on his effort in characterizing the lipid composition of human meibomian gland secretion from samples collected from 120 donor individuals by the combined use of different lipid mass spectrometry techniques.
Drs. Guan Xueli and Tushita Rupasinghe reported on macrophage lipid remodelling during bacterial infection and lipid profiles of erythrocytes following Plasmodium falciparum infection respectively, thus providing hints on mammalian cells responses to pathogen interaction.
The above mentioned contribution were supported by talks in workshop 2 and 3 on lipid nomenclature (Dr. Harald Kofeler), bioinformatic analysis of lipidomics data (Dr. Dominik Schwudke, Dr. Andrej Shevchenko), Biocuration of lipid databases (Prof. Edward Dennis , a PostDoc from Iannis Xenarios laboratory). Several posters were also focused on similar topics. Among these I would like to mention the ones presented by Husna Begum on natural variations in human blood lipids, by Jacklyn Ruilin Chen on erythrocytes lipidomics in patients with Schizophrenia, and the one by Jing Yan Lim on Cell cycle lipidomics as examples of lipidome studies in health and disease.

Lipid imaging

This group of contributions includes studies of new methods to visualize lipids either in cells or tissues.

Prof. Robert Parton reported on the setup for an electron-based method to map phosphatylserine, phosphatidylethanolamine, and ceramide at the subcellular level.

Dr. Andreas Zumbusch reported on the imaging capability of a Coherent anti-stokes Raman scattering based microscope on lipid droplets formation and dynamics without the use of any dye.

Dr. Brendan Prideaux reported on his MALDI imaging approach to lipid localization on lungs of rabbits infected with Mycobacterium tuberculosis.

Dr. Christian Eggling reported on the use of a super-resolution STED microscope for the study of different lipids diffusion properties on cell plasma membrane.

Among the abstracts the one by Dr. Shareef Ismail dealt with the possibility to apply orthogonal chemistry to myo-inositol with the perspective to visualize inositol containing lipids in yeast.

Lipidomics and functional studies in non-vertebrate organisms

A number of talks and poster presentations were related to lipid analysis in non-vertebrate eukaryotes and prokaryotes. Less complex organisms indeed provide on one side, simplified models for the study of complex biological phenomena, on the other the comparison of lipidomes from different organisms might add on DNA and protein comparisons for evolutionary studies.

On this topic Dr. Xun Huang presented data from his studies on lipid storage in Drosophila and C. elegans, Dr. Joanne Yew presented data on pheromones from insects, Drs Yuki Nakamura, John Harwood, Neil Clarke, and Chew Fook Tim presented data on algal lipids, while Dr. Ivo Feussner reported on galactolipids in Arabidopsis Taliana. Among the posters on these topics I found of special interest the one by Dr. Julio Sampaio on Drosophila lipidomics under different diet conditions and at different developmental stages.

Functional studies

This group of contributions includes studies aimed at understanding lipid metabolism regulation and lipids function. The topic was already introduced by workshop 1 where Prof Song Baoliang and Prof Scott Summers discussed about Cholesterol and Ceramide metabolisms as paradigmatic examples of molecularly resolved regulated lipid pathways.

Dr. Guillame Thibault reported on the unfolded protein response (UPR) triggered by experimentally induced phospholipid imbalance and on the fact that UPR restores sub-cellular organelles functions leading to cell adaptation to the "new" lipid composition.

Dr. Herman Overkleeft reported on the development of new inhibitors for glycosphingolipid synthesis and degradation as well as on new ways to measure glycosphingolipid catabolic reactions in living cells.

Dr. Toon de Kroon reported on the discovery of a novel pathway for the regulation of fatty acid desaturation in yeast phospholipids.

Dr. Xu Chenqui reported on the discovery of a regulatory function of membrane phospholipids in T cell receptor signalling regulation.

Dr. M. Blanc reported on the link between viral infection, interferon response and 25-hydroxysterol generation. 25-hydroxysterol was also shown to exert antiviral activity at physiological levels.

Among the different posters focused on functional aspects of lipid biology I would like to mention that by Dr. Marielle Klein approaching the ceramidome response and participation to toll-like receptor signalling.

Membrane dynamics studies

A smaller number of contributions and posters described biophysical approaches to the study of membrane dynamics.

Dr. Patricia Bassereau reported on her studies on the behaviour of different lipid species and lipid-toxin interaction in Giant Unilamellar Vesicles providing models of membrane curvature-induced lipid sorting.

Dr. Adam Orlowski reported his experience in membrane behaviour atomic simulation and the effect of cholesterol on transmembrane protein domain hydrophobic matching. Among the posters it is worth mentioning the one by Kamila Oglecka on the dynamics of lipid rafts in Giant Unilamellar Vesicles.

Protein-lipid interaction studies

Few talks were also devoted to studies approaching the issue of protein-lipid interaction. The first by Dr. Anne-Claude Gavin on a systematic approach to the identification of lipid binding specificities of selected protein domains from yeast proteins. Dr. Ajay Mahalka reported on his studies on HSP90 interaction with lysosomal lipids, among which BMP. Dr. Lok Hang Mak reported on the synthesis of a small molecule mimicking the PH domain of PLC-delta for the binding to PtdInd4,5P2.

Assessment of the results and impact of the event on the EUROCORES Programme

The 4th ISLS has been a meeting extremely relevant for the EUROCORES Programme and especially for the euro MEMBRANE group as it was extremely focused on the leading edge technologies in membrane lipids analysis. The meeting provided an interesting environment for lipid specialists to discuss their data and to acquire knowledge on innovative approaches for the studies of membrane biology. The discussion sessions were extremely intense and useful to young and established scientists. A further important aspect of this meeting was that linked to the fact that attracting a significant number of lipid biology specialists it has provided a fruitful ground for the establishment of collaboration among labs which were not in contact before.

Molecular mechanism of metabolic branching in glycosphingolipid metabolism

Institute of Protein Biochemistry
National Research Council of Italy

Giovanni D'Angelo, PhD

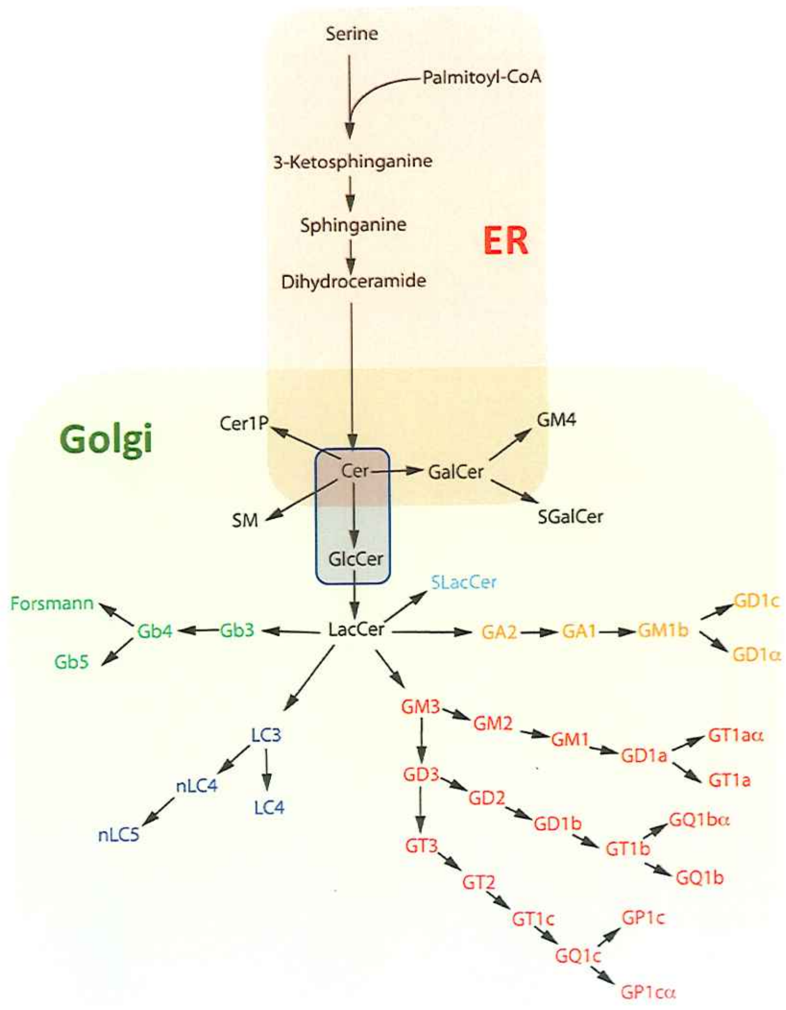
4th International Singapore Lipid Symposium

March 13–16, 2012

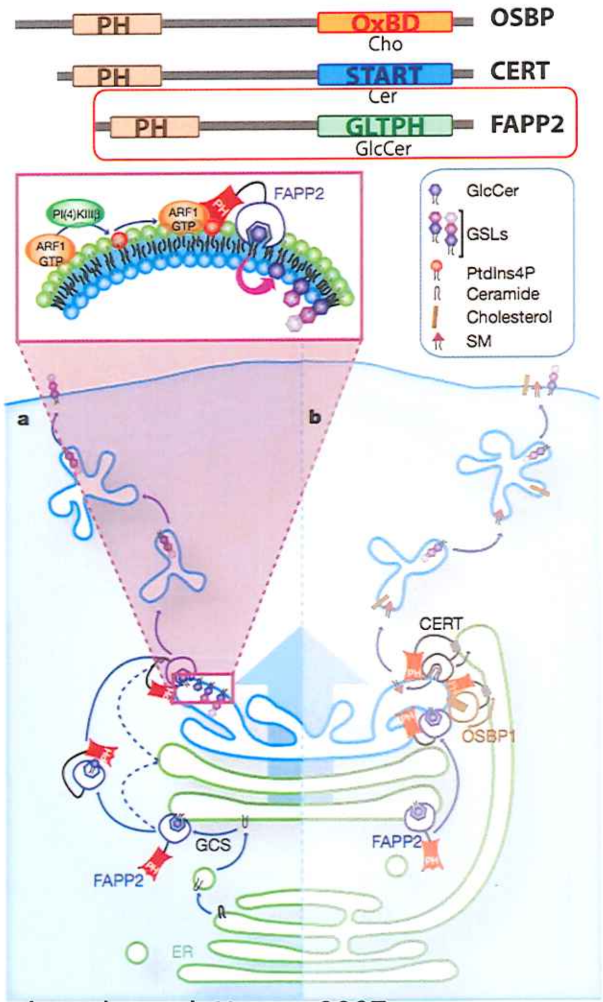
National University of Singapore

Center for Life Sciences

Glycosphingolipid Metabolism



Lipid Transfer Proteins



D'Angelo et al. Nature 2007

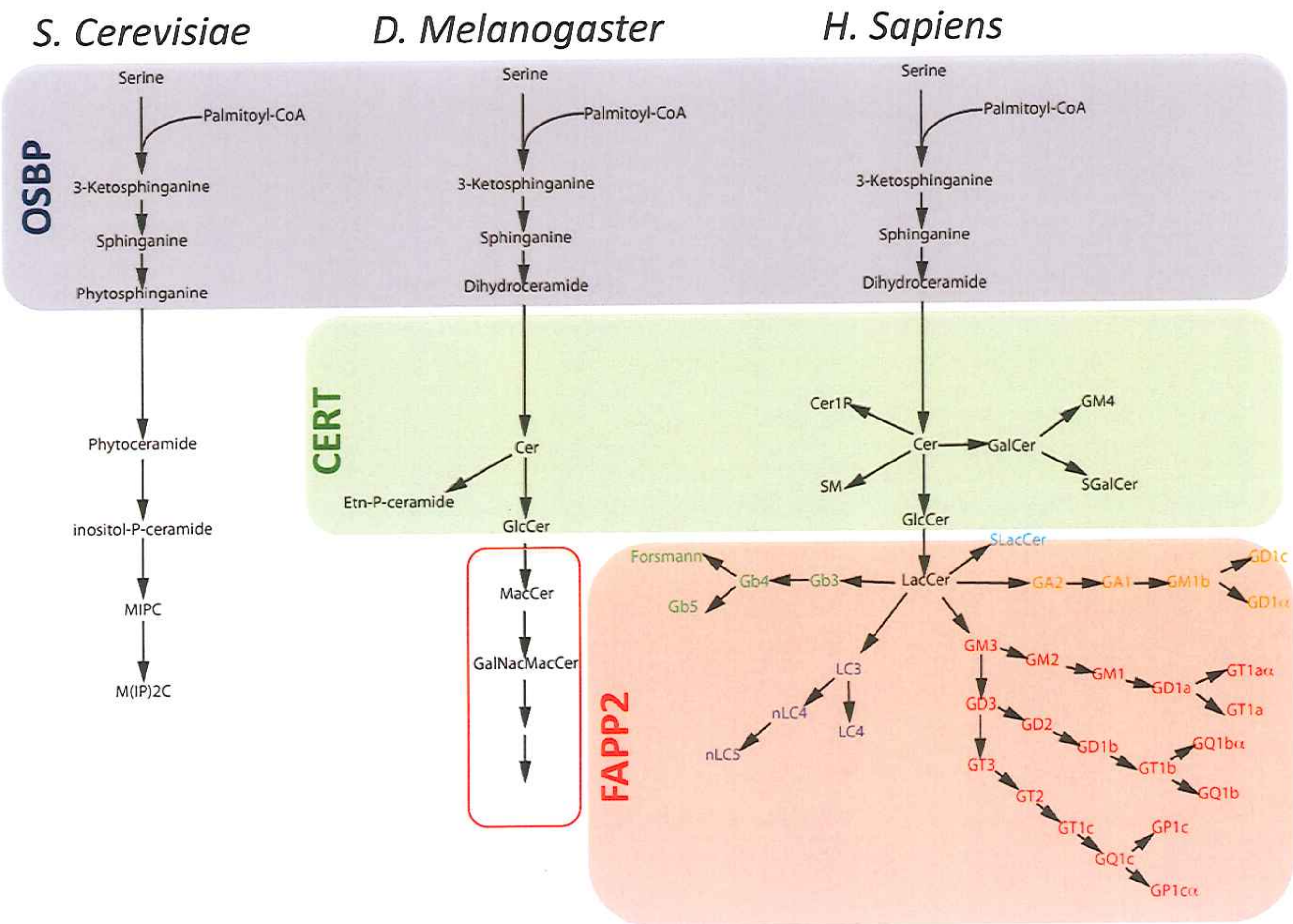
FAPP2 is a Golgi associated protein able to transport GlcCer across the Golgi in a non-vesicular fashion.

GSL synthesis downstream GlcCer requires FAPP2

Why?

How?

LTPs in evolution



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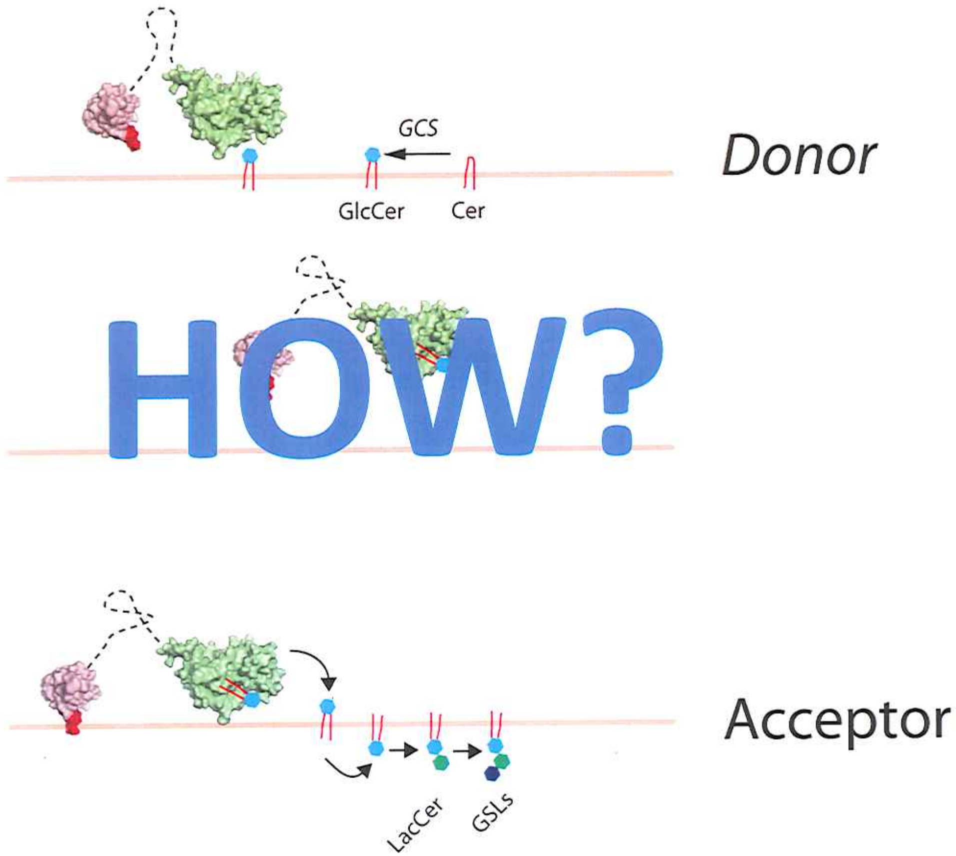

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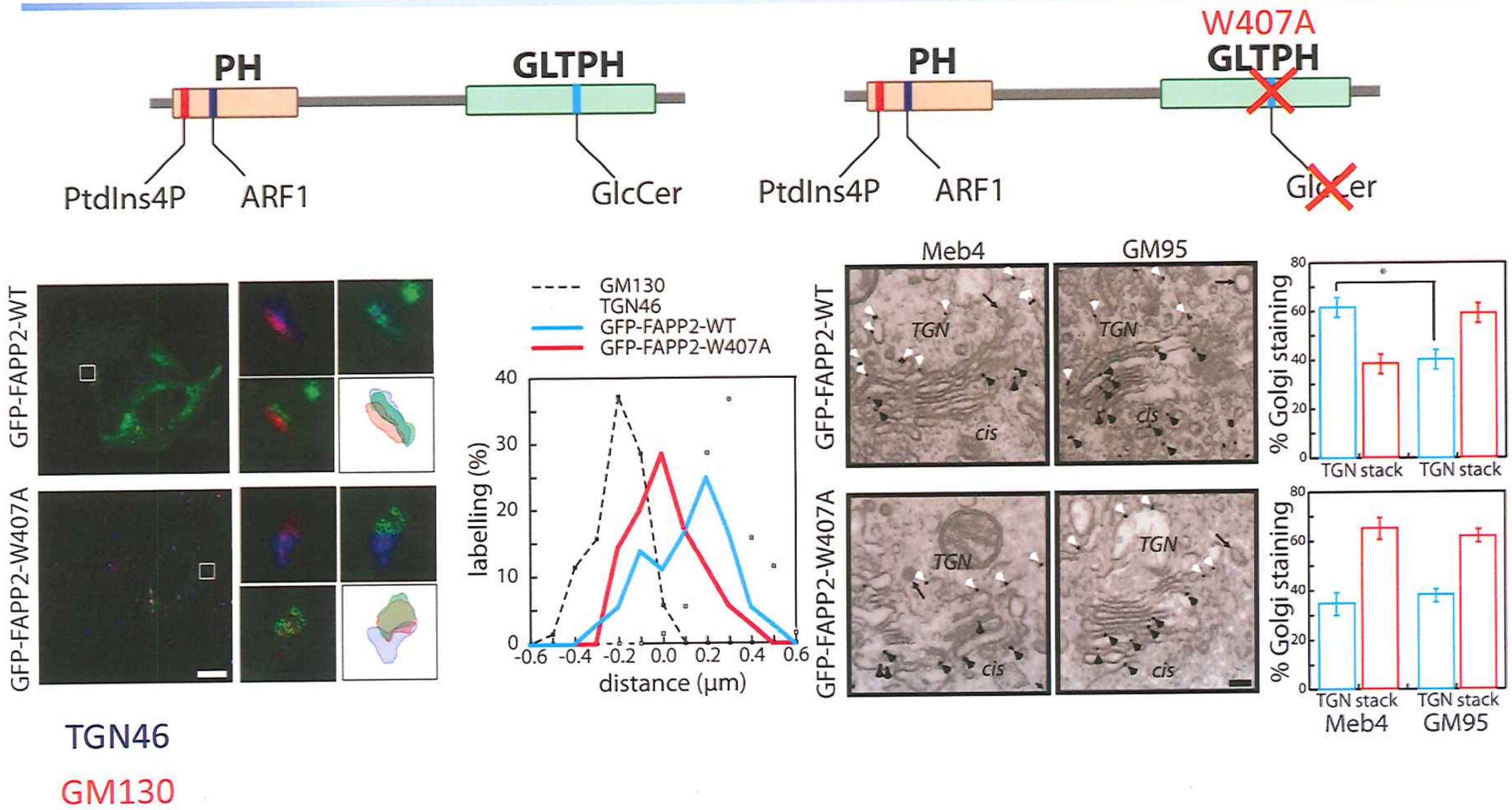
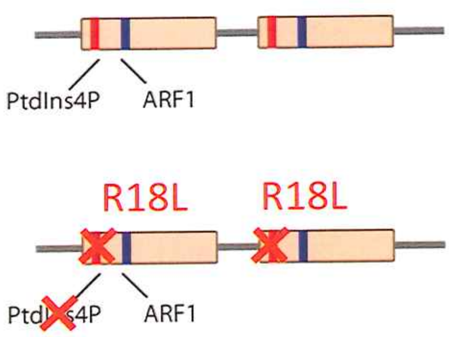
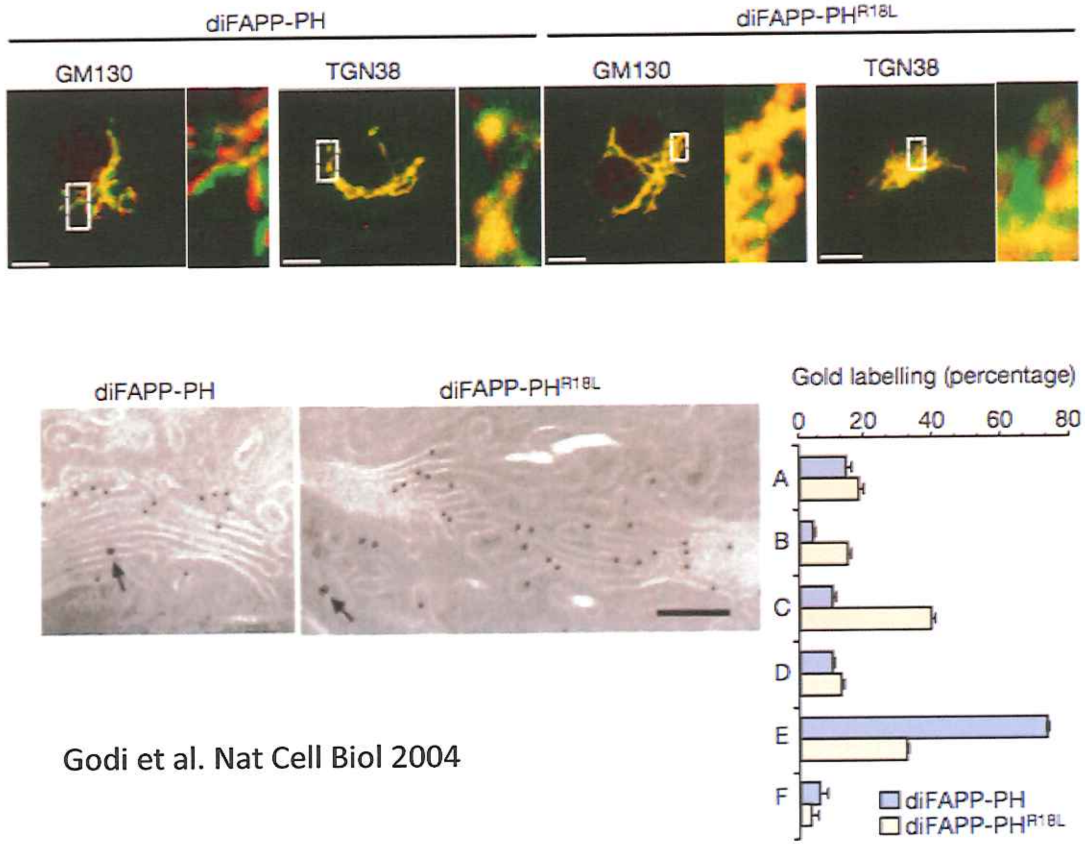


PH
GLTPH
PtdIns4P
ARF1
GlcCer
PH
W407A
GLTPH
PtdIns4P
ARF1
GlcCer
GFP-FAPP2-WT
GFP-FAPP2-W407A
TGN46
GM130
GM130
TGN46
GFP-FAPP2-WT
GFP-FAPP2-W407A
labelling (%)
distance (µm)
Meb4
GM95
TGN
cis
% Golgi staining
TGN stack
TGN stack
Meb4
GM95

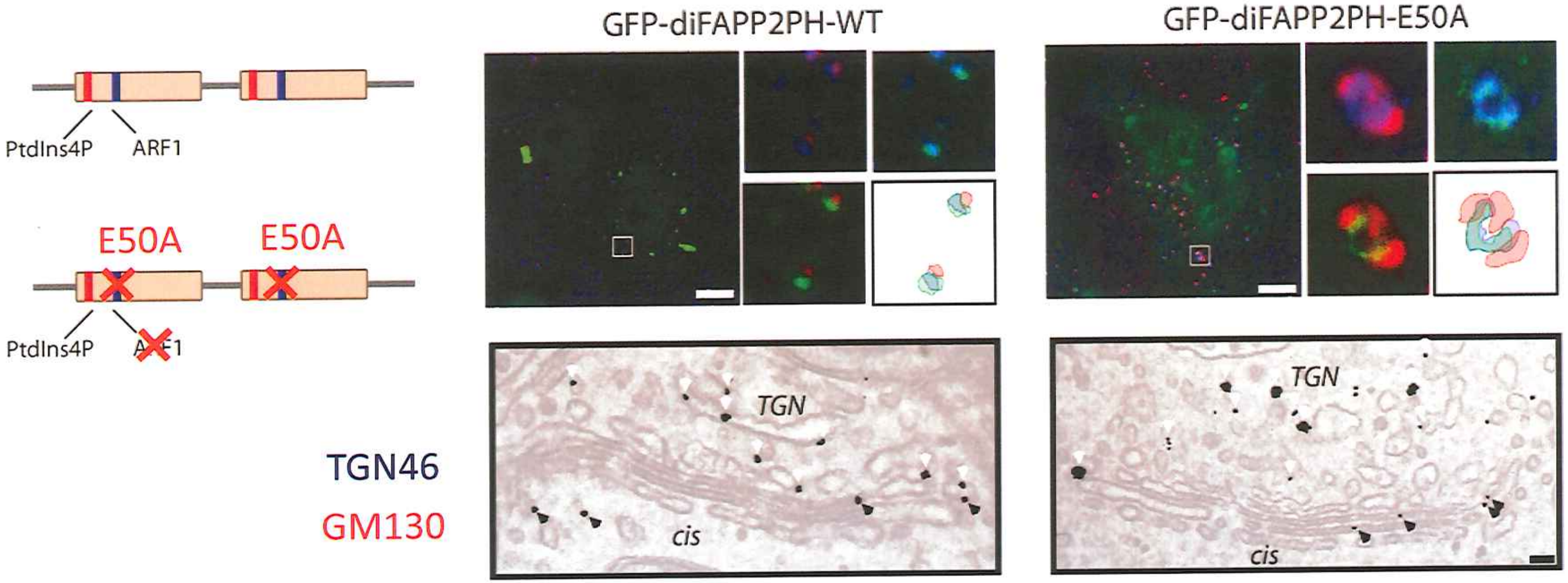
FAPP2





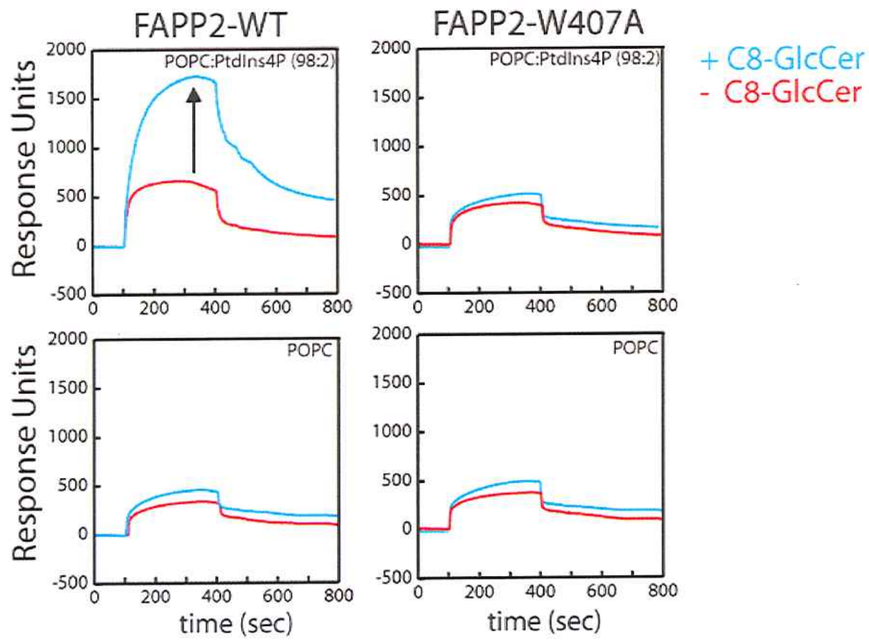
Godi et al. Nat Cell Biol 2004

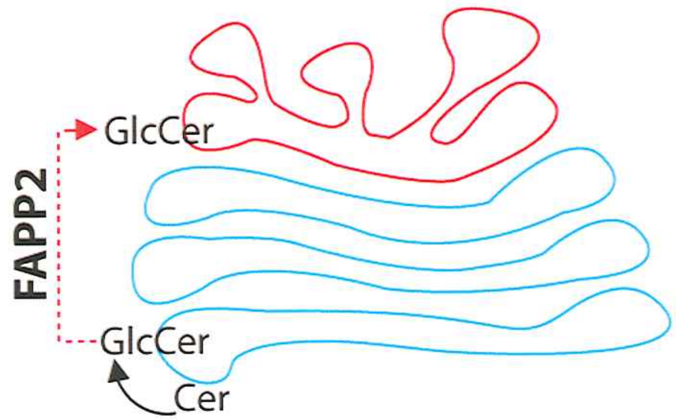
FAPP2



PtdIns4P determines FAPP2 enrichment at the TGN

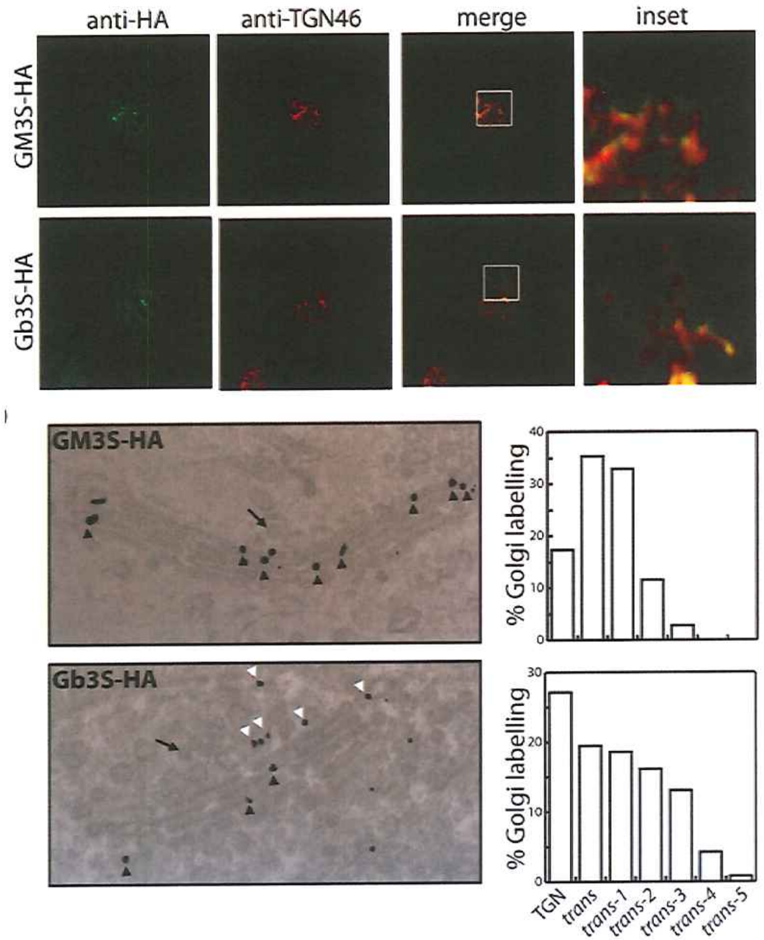
FAPP2

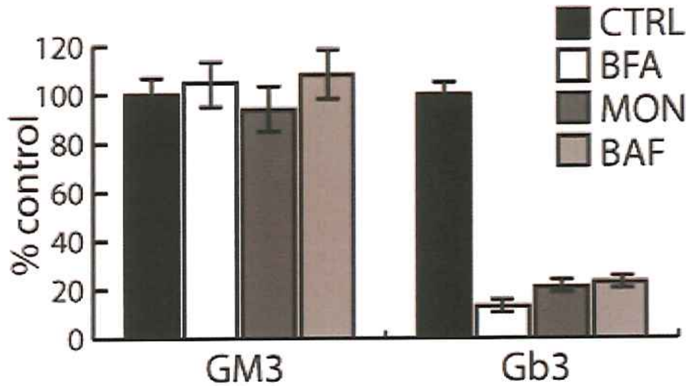


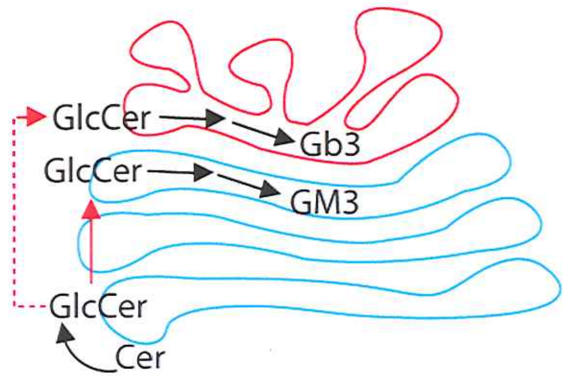


- GlcCer Binding increases the probability of FAPP2 to be located at the TGN
- GlcCer Binding increases FAPP2 affinity for PtdIns4P

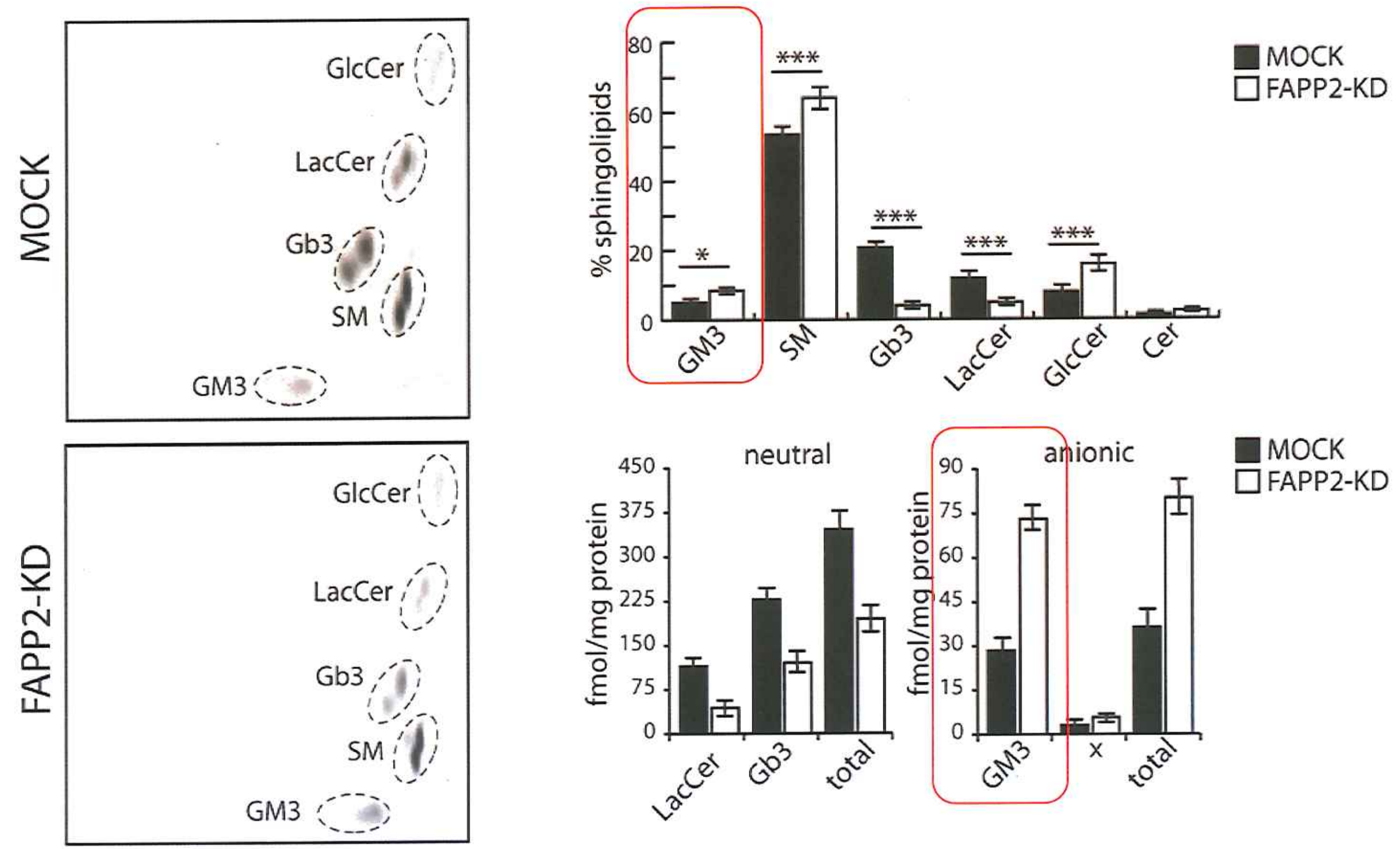
GSL synthesizing enzymes



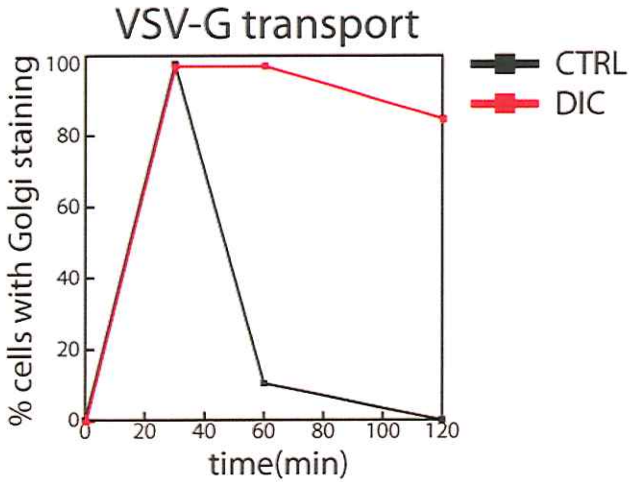


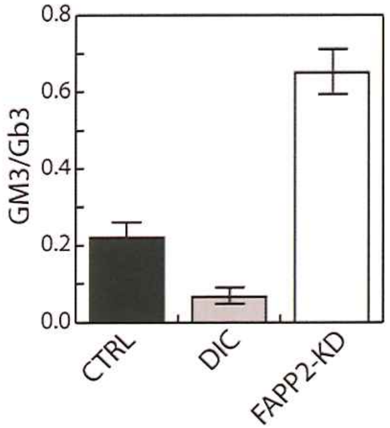


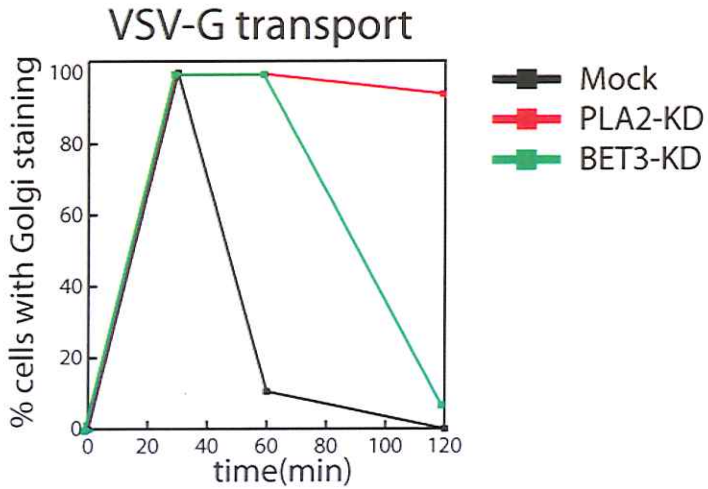
FAPP2 role in GSL synthesis

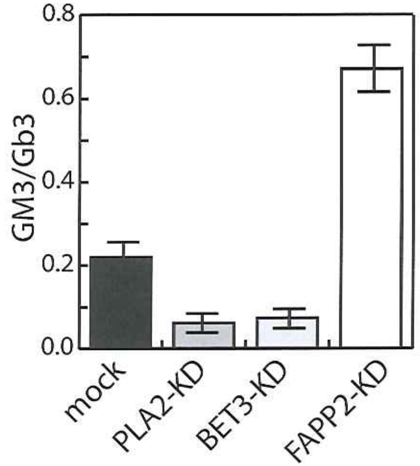


Membrane trafficking of GSLs

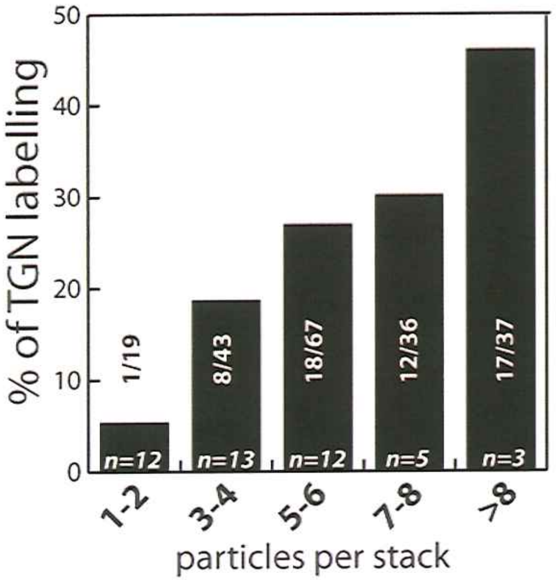








FAPP2 GM3S



% of TGN labelling
50
40
30
20
10
0
1/19
8/43
18/67
12/36
17/37
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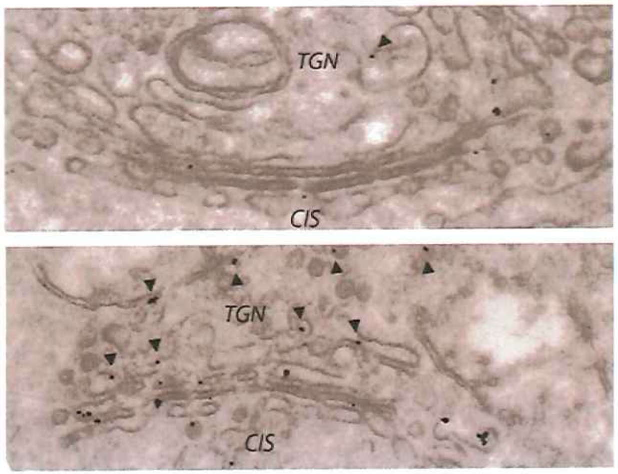
n=3
1-2
3-4
5-6
7-8
>8
particles per stack



TGN
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TGN
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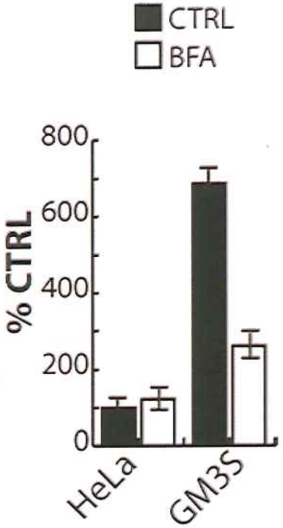

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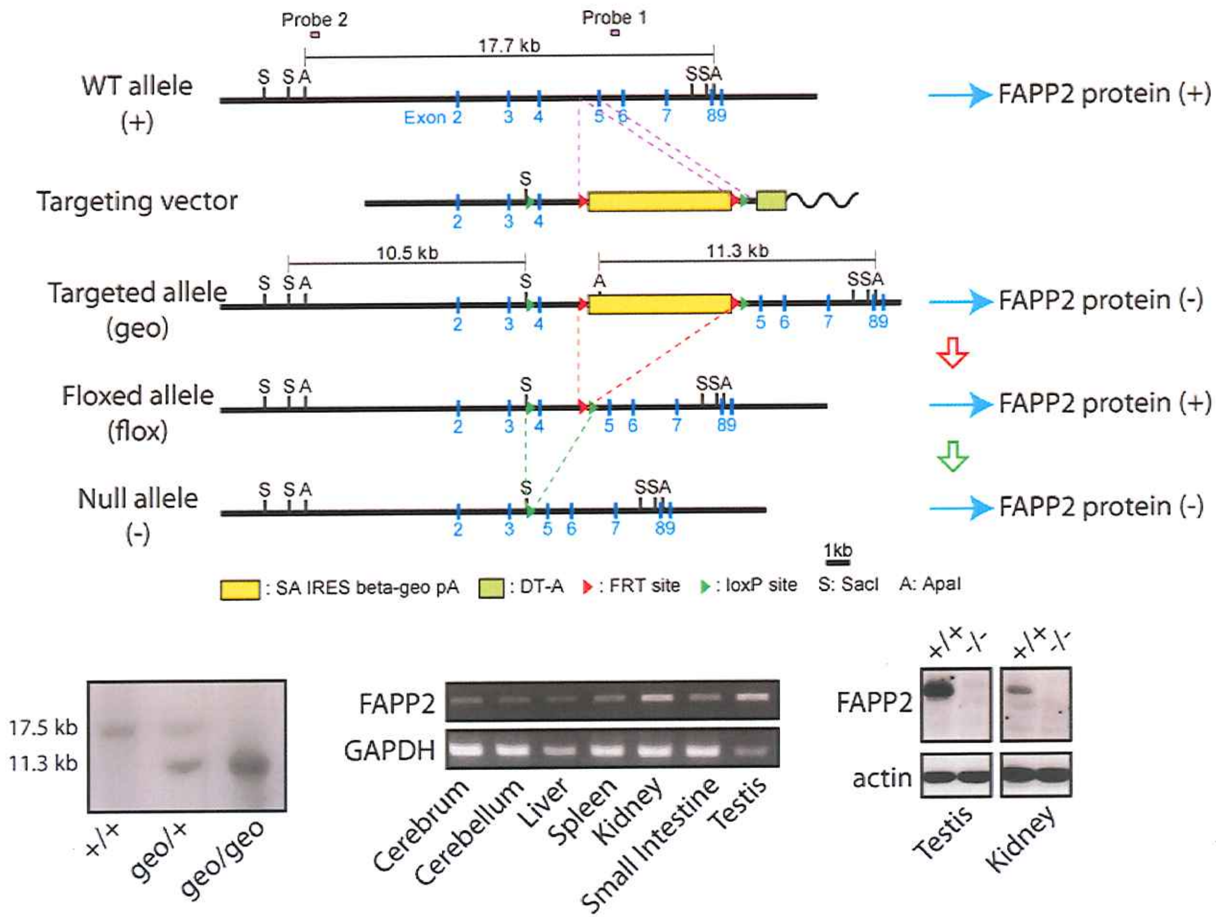
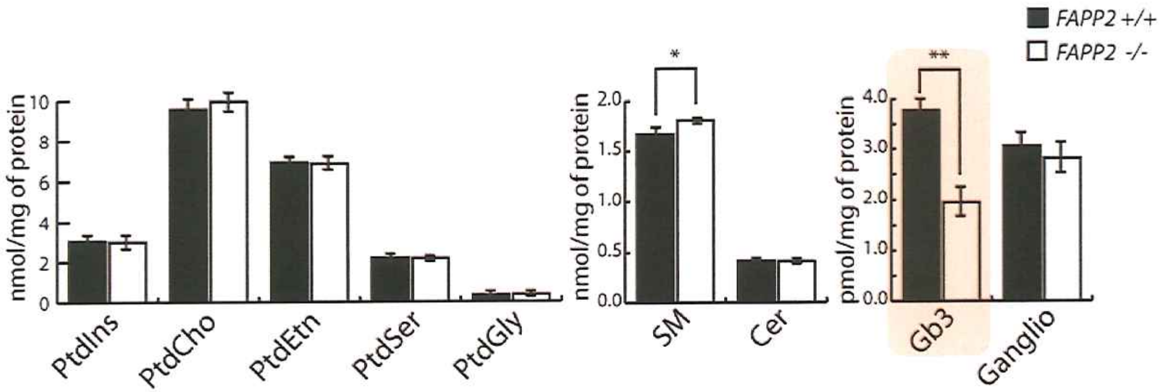
HeLa
GM3S

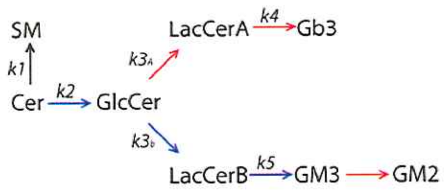
FAPP2-KO mice

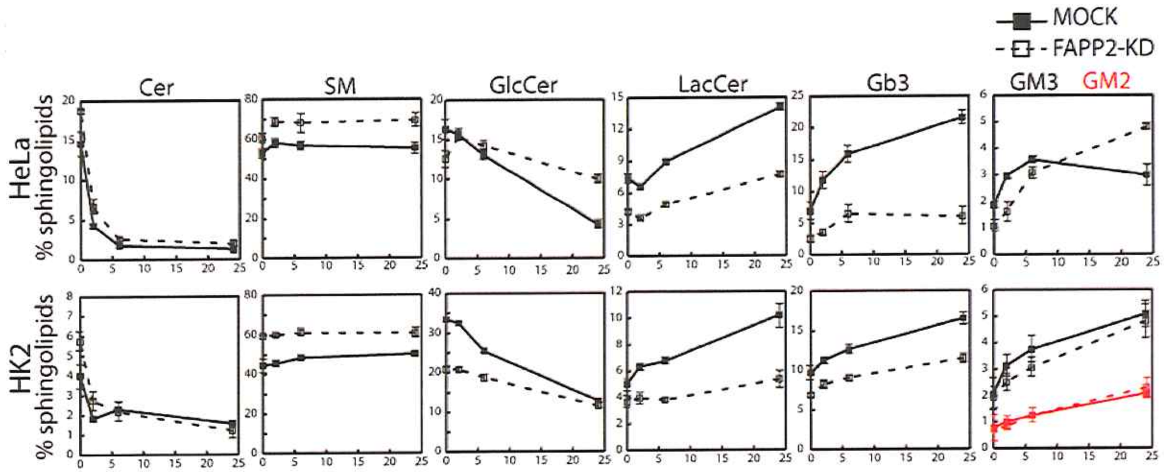


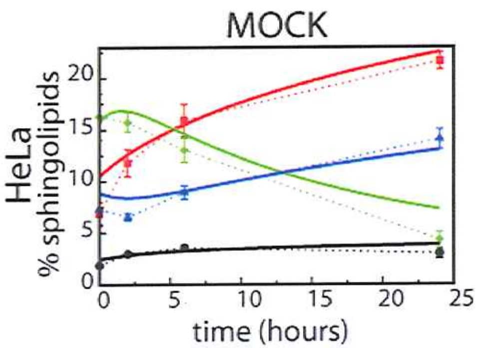
FAPP2-KO mice

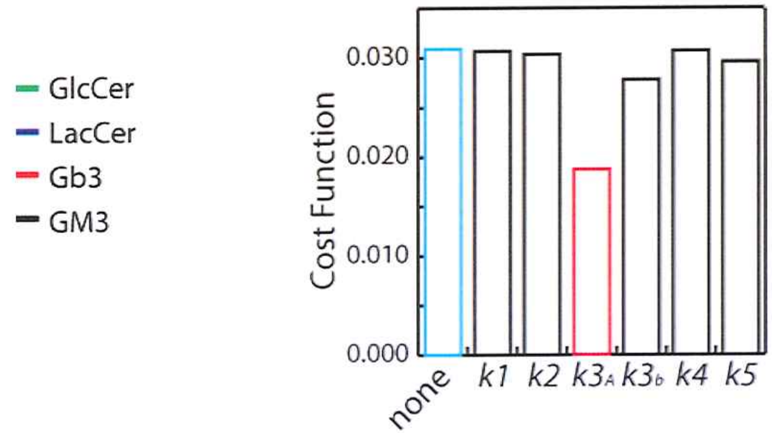


FAPP2 role in GSL metabolic branching

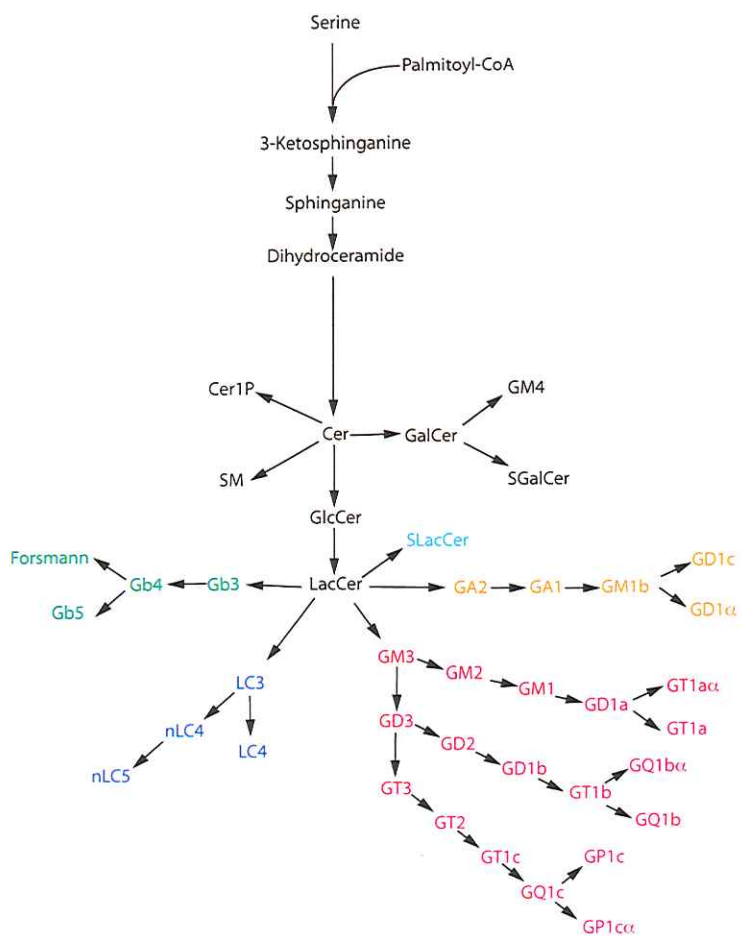


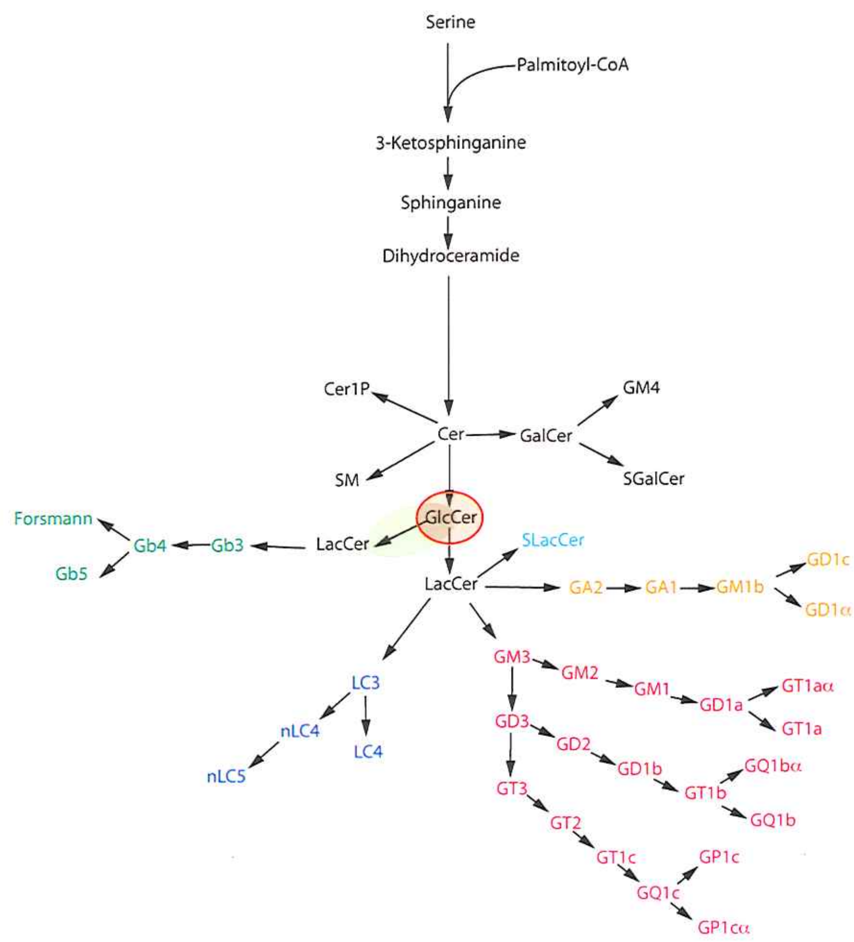




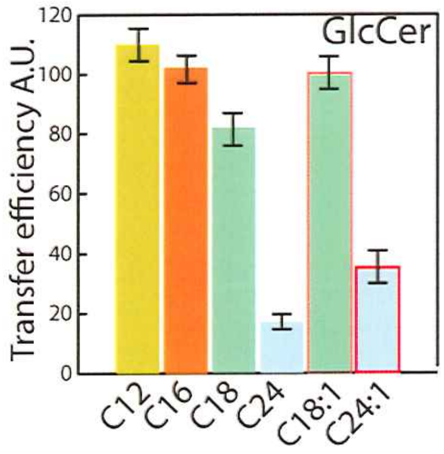


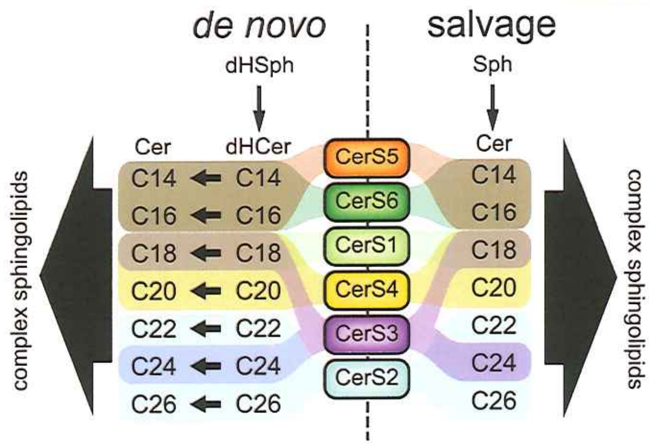
FAPP2 role in GSL metabolic branching

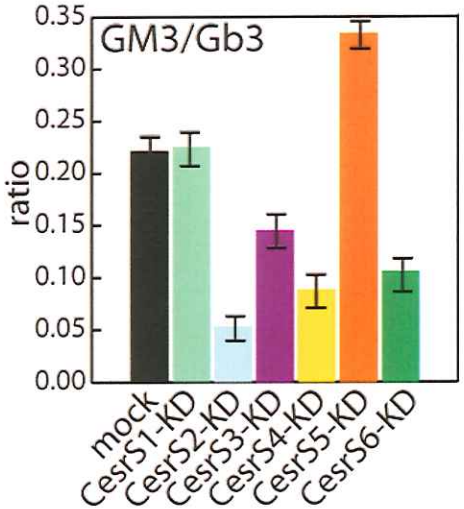


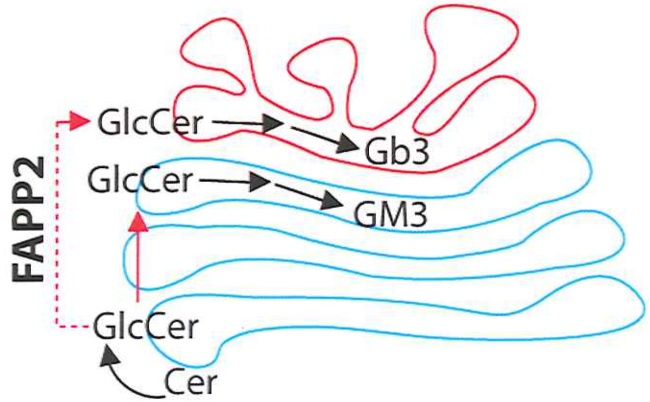


FAPP2 and GlcCer acyl chain length









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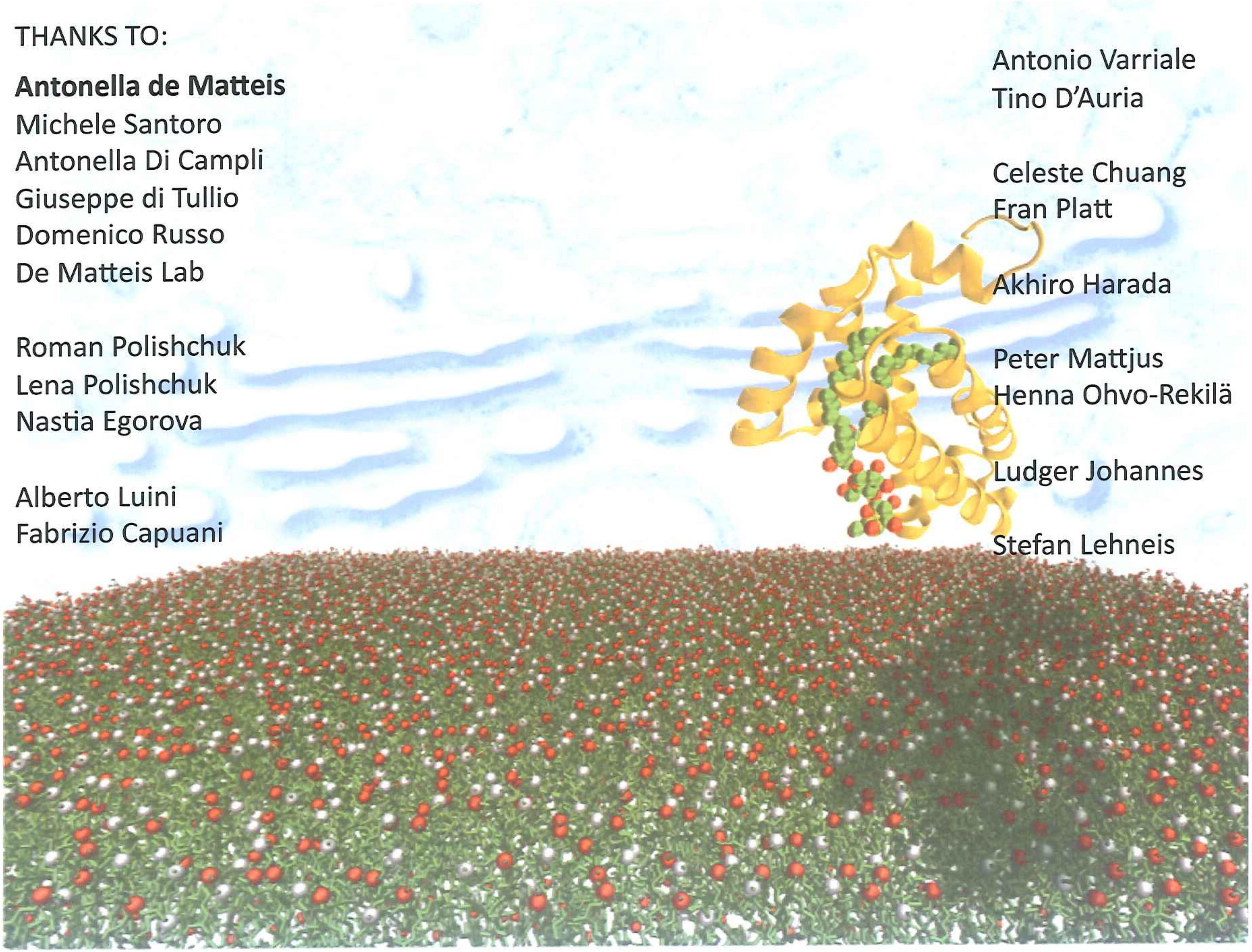
conclusions

- Vesicular and non-vesicular transport of GlcCer assign two different metabolic fates to this molecule
- FAPP2 is required for GSL synthesis at the TGN
- Vesicular or non vesicular transport of GlcCer are determined by its acyl chain length.

questions

- What is the physiological role of this mechanism
- How is this mechanism regulated
- What is the function of specific GSLs



THANKS TO:

Antonella de Matteis
Michele Santoro
Antonella Di Campli
Giuseppe di Tullio
Domenico Russo
De Matteis Lab

Roman Polishchuk
Lena Polishchuk
Nastia Egorova

Alberto Luini
Fabrizio Capuani

Antonio Varriale
Tino D'Auria

Celeste Chuang
Fran Platt

Akhiro Harada

Peter Mattjus
Henna Ohvo-Rekilä

Ludger Johannes

Stefan Lehneis