

Exercise 8

Question 1

Name where the following lipids are produced and which enzymes mentioned in the lecture are required for their transport towards other membranes.

Lipid	Location of synthesis	Transporter proteins
Ceramide	ER with the hydrophobic intermediated facing the cytosol	CERT to golgi membrane
Cholesterol	ER	OSBP transports to trans-golgi membrane
Glycosylceramide	cytosolic leaflet of the golgi membrane	FAPP2 to golgi membrane
Phosphoinositide	synthesized in the ER but the processing takes place in is designated cellular compartment	phosphatidylinositol transfer proteins
Phosphatic acids	ER	
PI4P	trans-golgi	OSBP1 transport to ER
Sphingomyelin	Golgi membrane	vesicular transport (not a protein)
Triacylglyceride	ER	

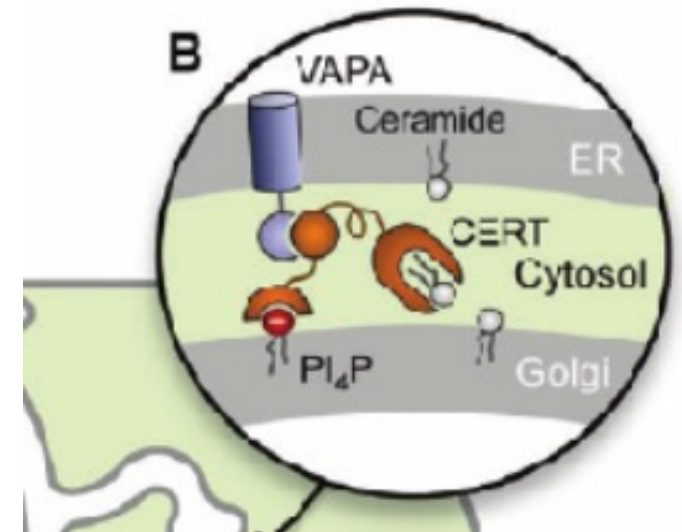
Question 2

The ceramide transporter CERT is located between the ER and trans golgi membrane and its activity can be inhibited with an inhibitor abbreviated HPA-12.

1. What is the effect on the ceramide levels in the ER and golgi membrane?
2. What is the effect of the CERT inhibition on the production of other lipids?
3. Is the lipid composition of other membranes effected by the inhibition of CERT?

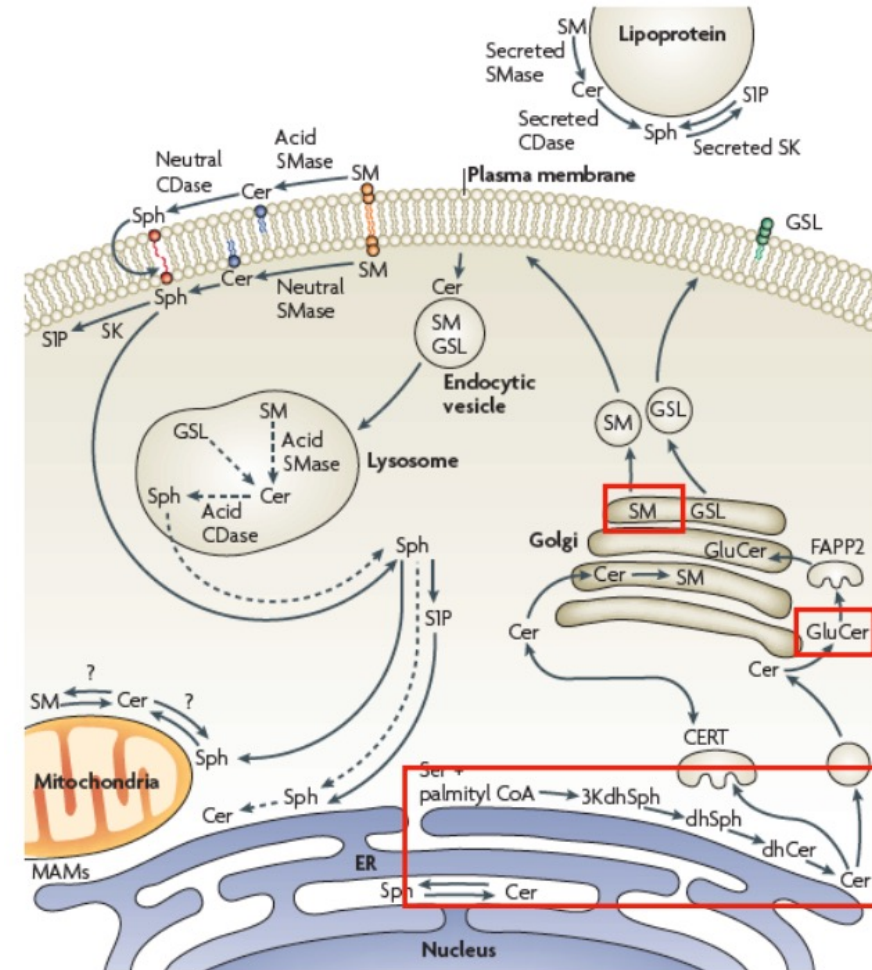
2.1. What is the effect on the ceramide levels in the ER and golgi membrane

The levels of ceramide will increase in the ER membrane since it will no longer be transported to the trans golgi membrane. Moreover, the production of ceramide will be inhibited in the ER due to the high levels of ceramide already present. The concentration of ceramide in the golgi membrane will decrease.



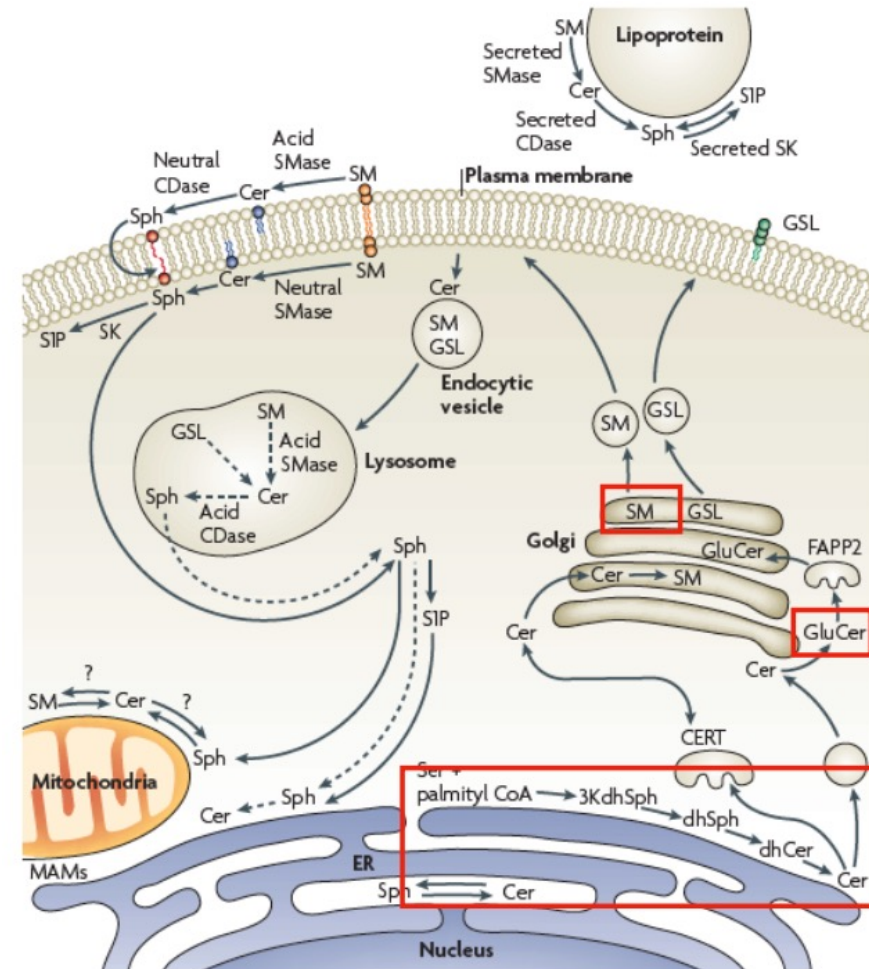
2.2. What is the effect of the CERT inhibition on the production of other lipids?

Since Ceramides will no longer be transported into the golgi membrane sphingomyelin will no longer be produced from ceramide. Ceramide can still be transported to the golgi membrane in vesicles for the production of glycosphingolipids.



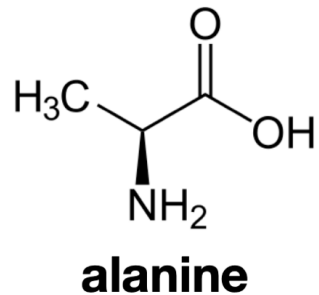
2.3. Is the lipid composition of other membranes effected by the inhibiton of CFRT?

From the golgi membrane the sphingomyelin is further distributed to the plasma mebrane and the lysosome where it can be futher metabolized. The inhibiton of CERT will therefore also have an effect on the plasma membrane and the lysosome membrane as well as the metabolizm within the lysosome. As SM will get metabolied also into other sphingolipids in the plasma mebrane also this lipid concentration will change.



Question 3:

In a genetic disease named hereditary sensory and autonomic neuropathy type I SPT subunits are mutated such that they catalyse preferential condensation of palmitoyl-CoA to alanine rather than serine.

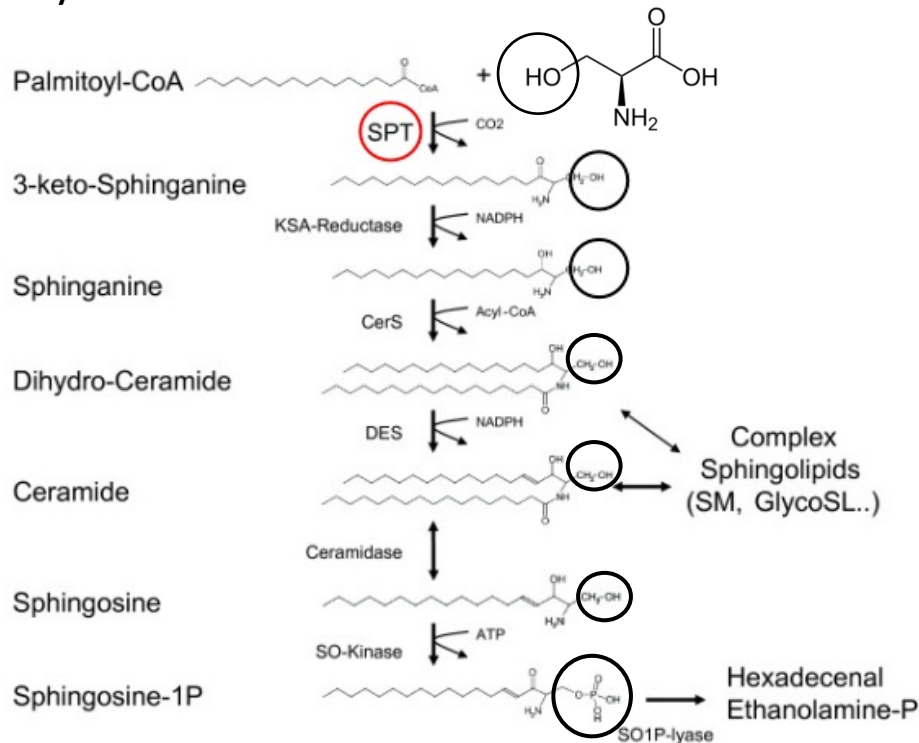


1. What is the metabolic outcome of this change in activity?
2. How is this propagated through the sphingolipid synthetic pathway?

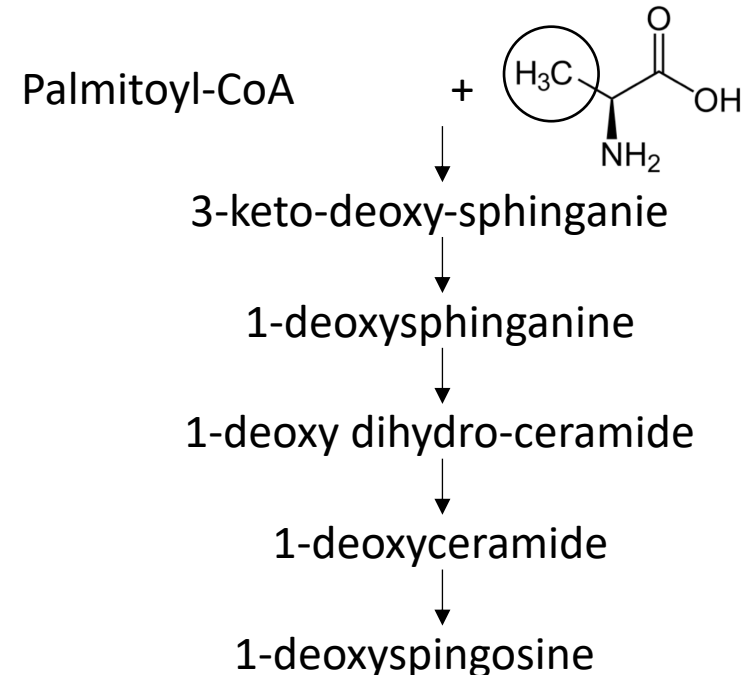
3.1. What is the metabolic outcome of this change in activity?

The difference between the serine which is used under healthy conditions for the synthesis of sphingolipids and alanine is the replacement of the OH group with a CH₃ group. This will be propagated withing the synthesis.

Healthy condition



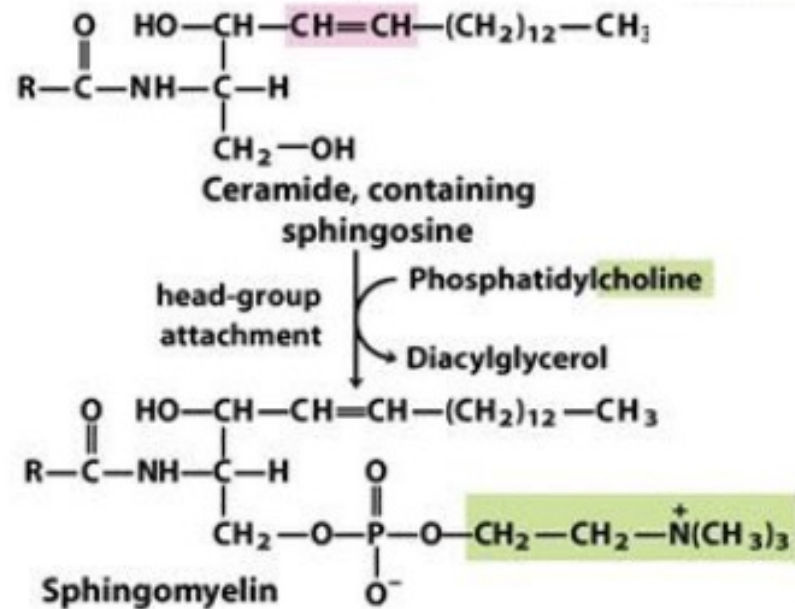
Gentic Disease



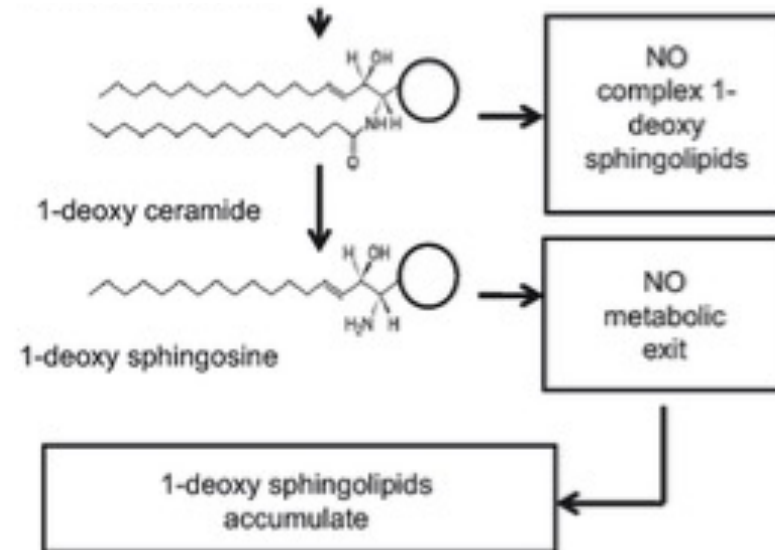
→ Phosphorilation is not possible

3.2. How is this propagated through the sphingolipid synthetic pathway?

In **healthy individuals** ceramide can be further processed and headgroups are enzymatically added. For example Sphingomyelin or ceramide-P-inositol can be formed like this.



In **individuals with the genetic disorder** 1-deoxy-ceramide can not be converted into more complex lipids and no headgroups can be added. Ceramide will be synthesized only via the salvage pathway and 1-deoxy sphingolipids will accumulate.

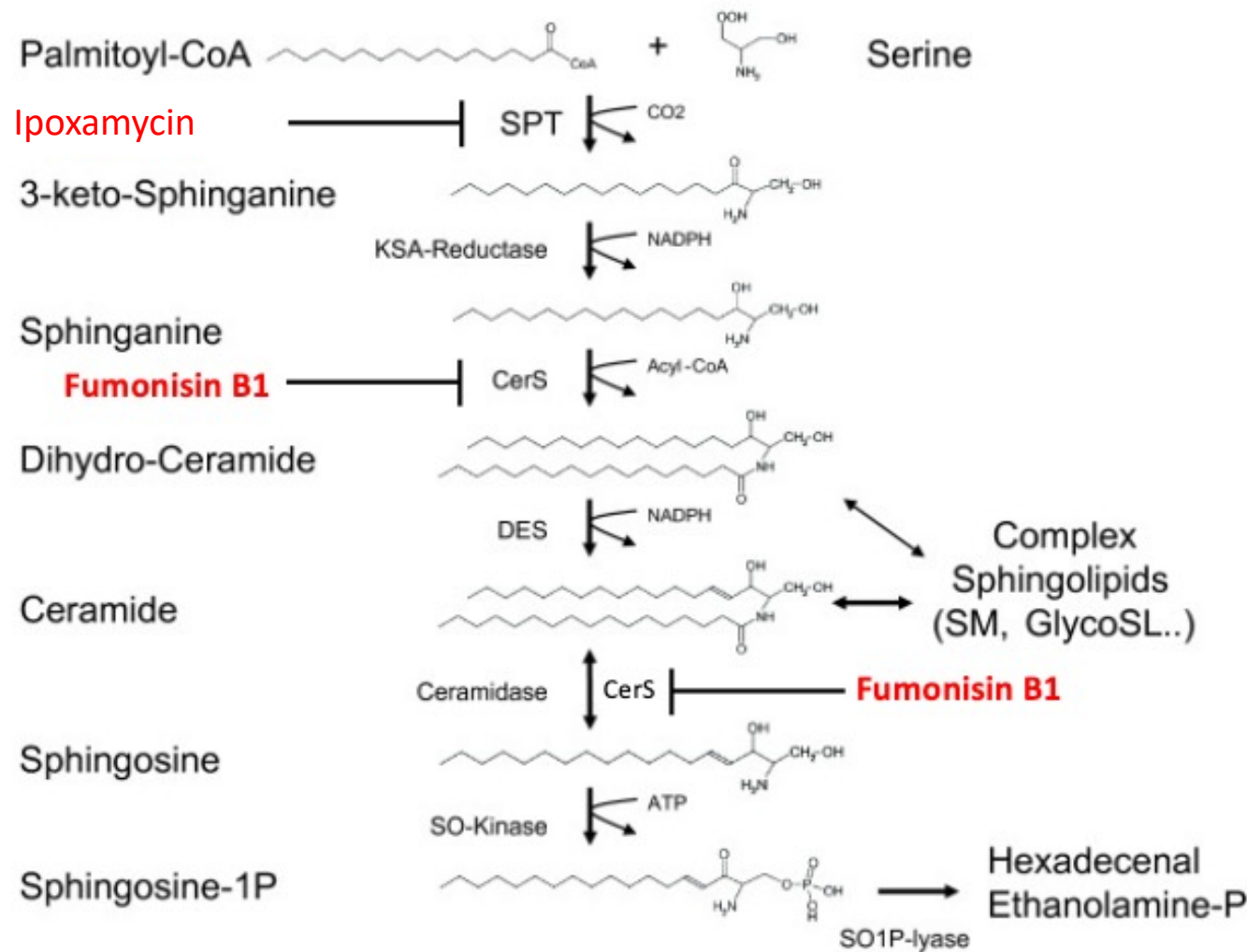


Question 4

The synthesis of sphingolipids can be blocked with the inhibitors ipoxamycin and fumonsin B1 which bind to the SPT and CerSes respectively.

1. For each inhibitor, which sphingolipid metabolites do you expect to be deprived and accumulated?
2. What treatment will lead to a faster disappearance of the cellular sphingomyelin pool?

Question 4 - 1 and 2



Ipoxamycin

- Decrease in ceramide and complex sphingo lipids
- Only a mild effect since only the de novo pathway is effected
- No accumulation of palmitoyl-Coa and serin → will be metabolized

Fumonisin B1

- Decrease in ceramide and complex spingolipids
- Increase in Sphinganine
- More efficient because it blocks the salvage pathway and the denovo synthesis of ceramide

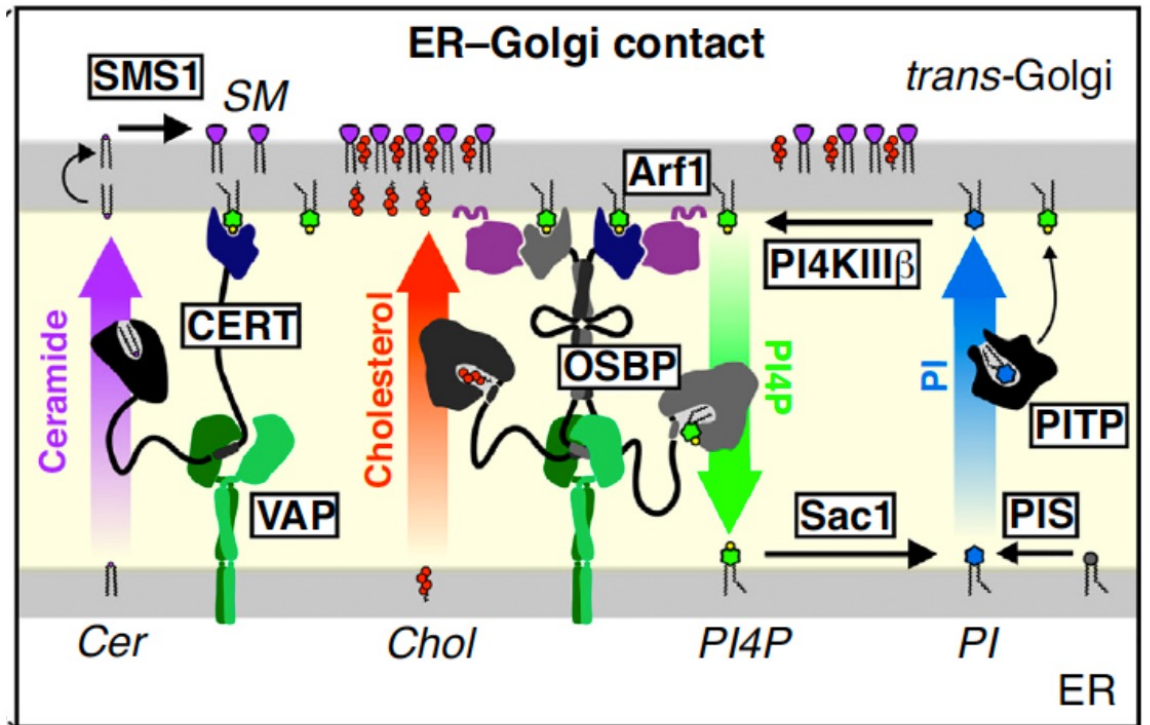
Question 5

OSBP1 is an important cholesterol/ PtdIns(4)*P* transfer protein at the ER-trans Golgi MCS. Predict the effects of OSBP1 inhibition on:

1. phosphoinositide levels at the ER and Golgi
2. localisation of CERT1 and FAPP2
3. sphingolipid metabolism

Question 5: OSBP1 is an important cholesterol/ PtdIns(4)P transfer protein at the ER-trans Golgi MCS. Predict the effects of OSBP1 inhibition on:

1. Phosphoinositide levels at the ER and Golgi
 - OSBP1 inhibition will lead to reduced phosphoinositide levels in the ER membrane and higher phosphoinositide levels in the golgi membrane.
2. Localisation of CERT1 and FAPP2
 - Even at higher concentrations of PI4O at the Golgi membrane Cert and FAPP2 can continue transferring Cer and GlcCer from the ER to the golgi and to the trans golgi membrane respectively.
3. Sphingolipid metabolism
 - CERT1 gets recruited to the membrane by PtdIns(4)P. Higher concentrations of PtdIns(4)P will therefore also increase the transfer for Ceramide to the trans-golgi and thereby the sphingolipid synthesis.



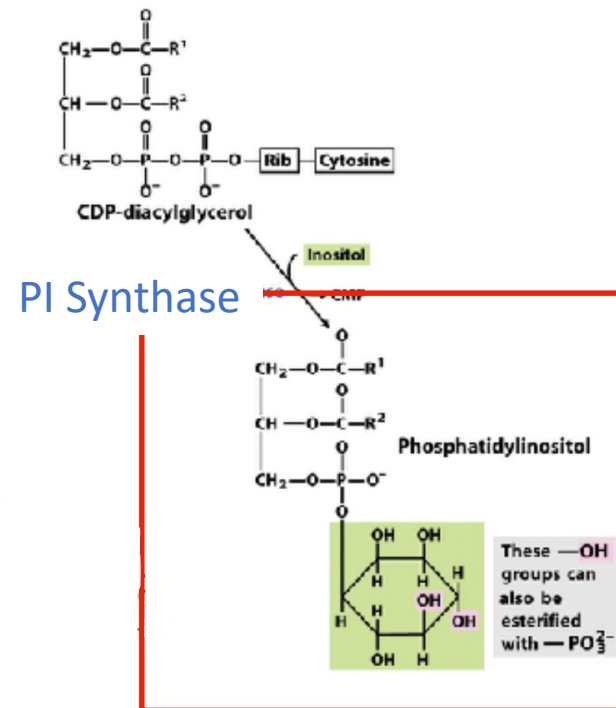
Question 6

The inhibitor L-690.488 is used in research to study the phosphatidylinositol synthase by inhibiting the enzyme.

1. What step of the PI synthesis synthesis will be inhibited?
2. What is the effect of a reduced PI level on
 - a. Membrane composition and recognition
 - b. Cellular signaling

6.1. What step of the PI synthesis synthesis will be inhibited?

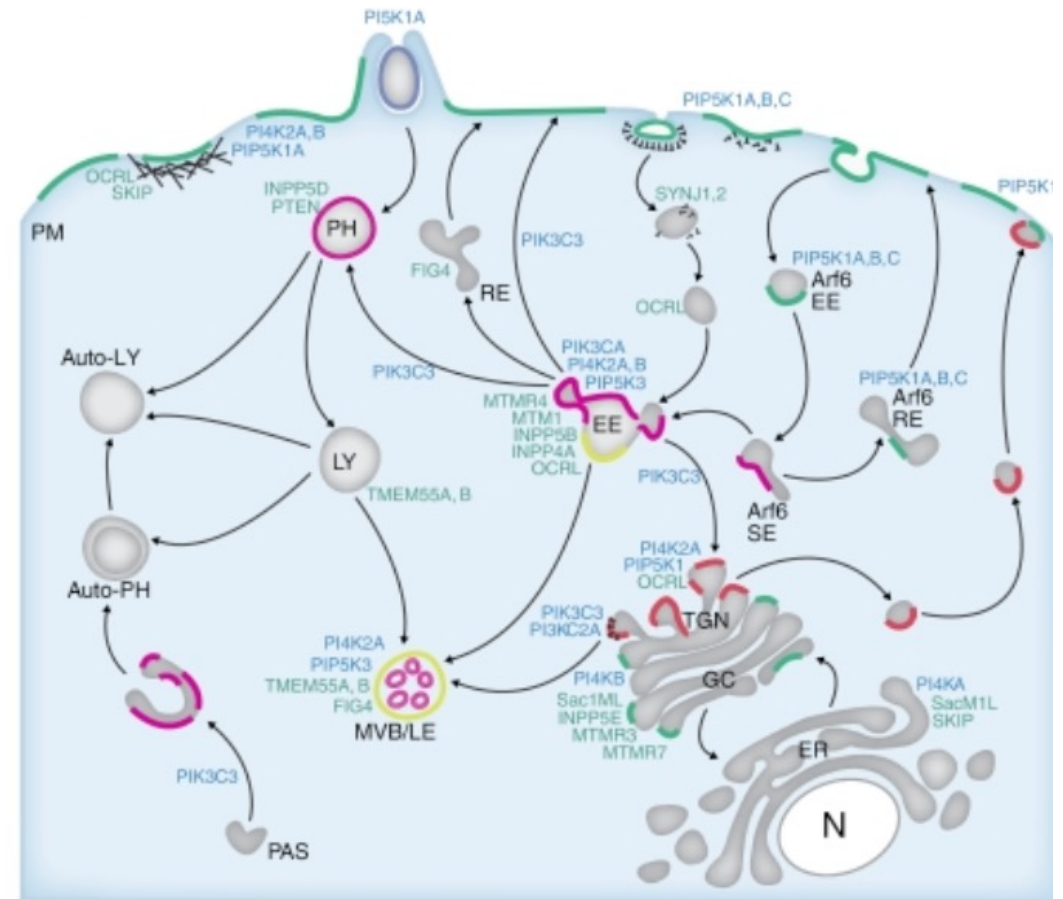
→ The condensation of CDP-diacylglycerol and inositol will be inhibited.



2.1. What is the effect of reduced PI level on

a) Membrane composition and recognition

- Less PI is trafficked to other cells where it would normally get phosphorylated into membrane specific PI.
- Less PI and membrane specific PI
- Many proteins recognize PI in the membrane. The proteins may therefore no longer recognize the membrane and bind the cell.



2.1. What is the effect of reduced PI level on

b) Cellular signaling

- PI plays an important role in cellular signaling.
- The blocking of PI will therefore inhibit specific signaling paths and lead to alternated cell proliferation and cell death.

