Welcome to BCII lesson 9

Chimie Biologique II

Biological Chemistry II

BIO-213

Teacher

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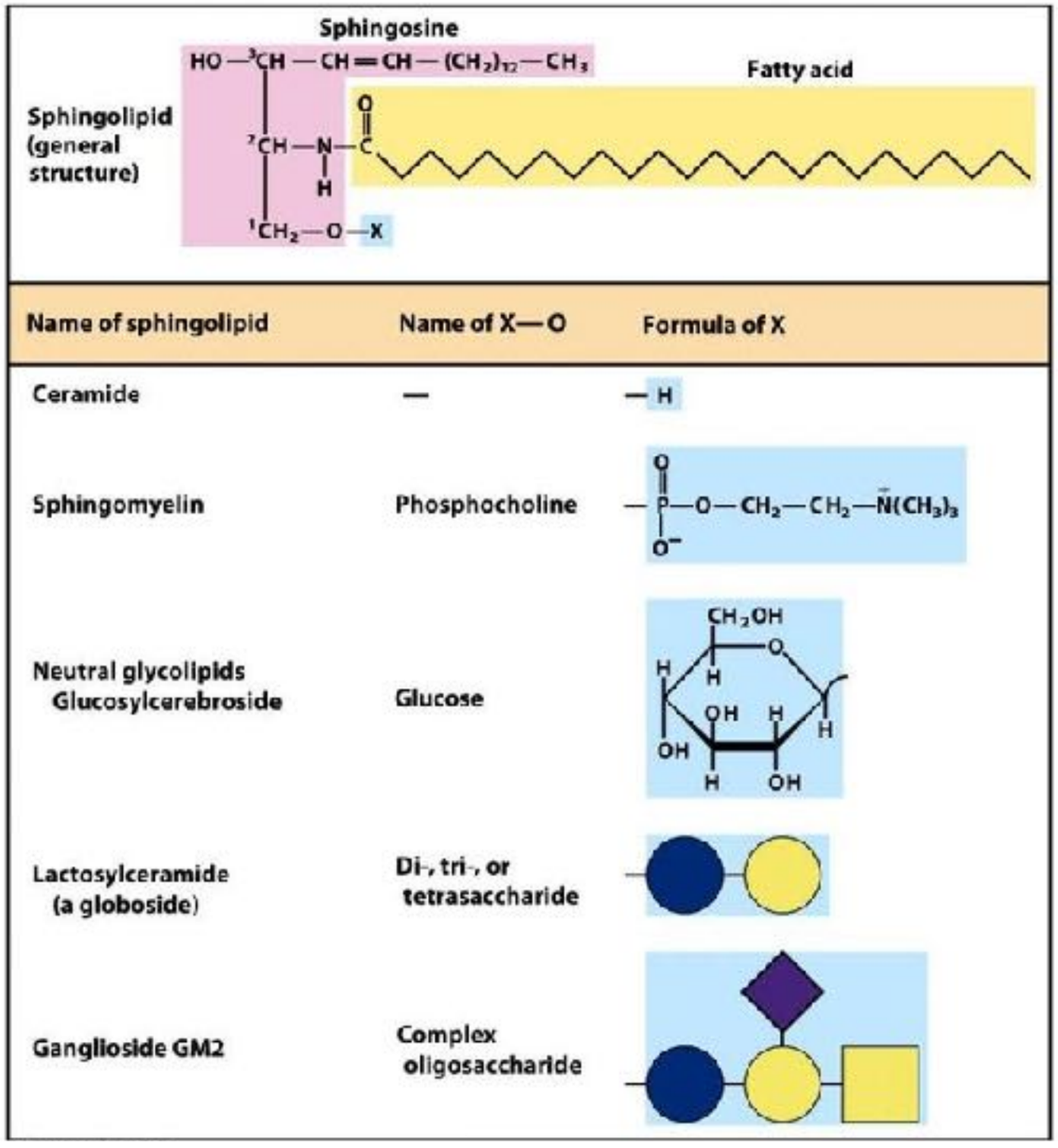
Lecture 9

The biosynthesis of lipids 2

- Sphingolipids
- Phosphoinositides

Sphingolipids



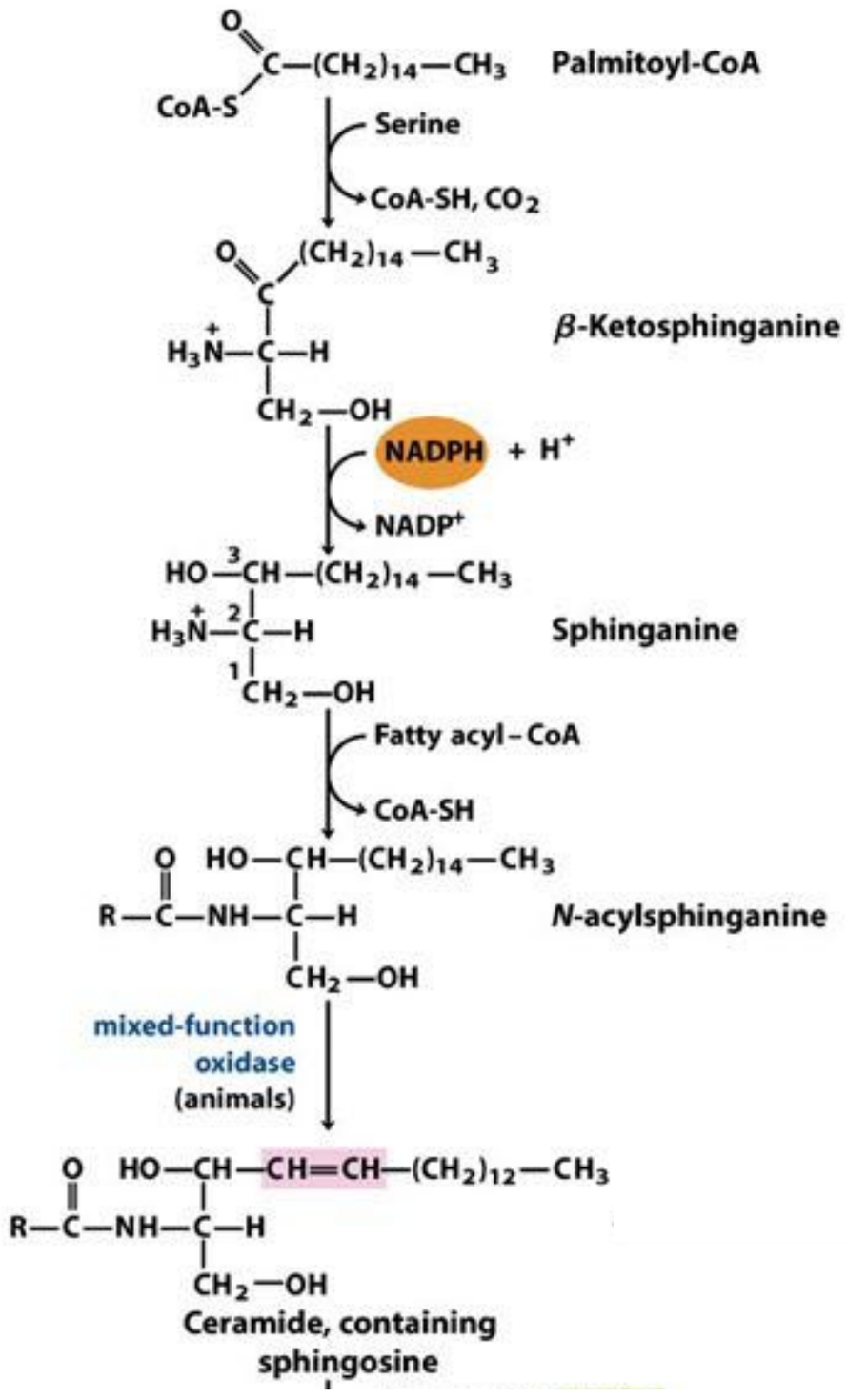
Sphingolipids are a class of lipids containing a backbone of sphingoid bases, a set of aliphatic amino alcohols that includes sphingosine.

The common backbone to all complex sphingolipids is **ceramide** that can accept numerous polar head groups.

The most abundant sphingolipid in many mammalian cell types is **sphingomyelin** whereby the ceramide backbone is complexed to a phosphocholine head group.

Ceramide can be variably glycosylated to produce a vast array of **glycosphingolipids**

Sphingolipids-Ceramide synthesis

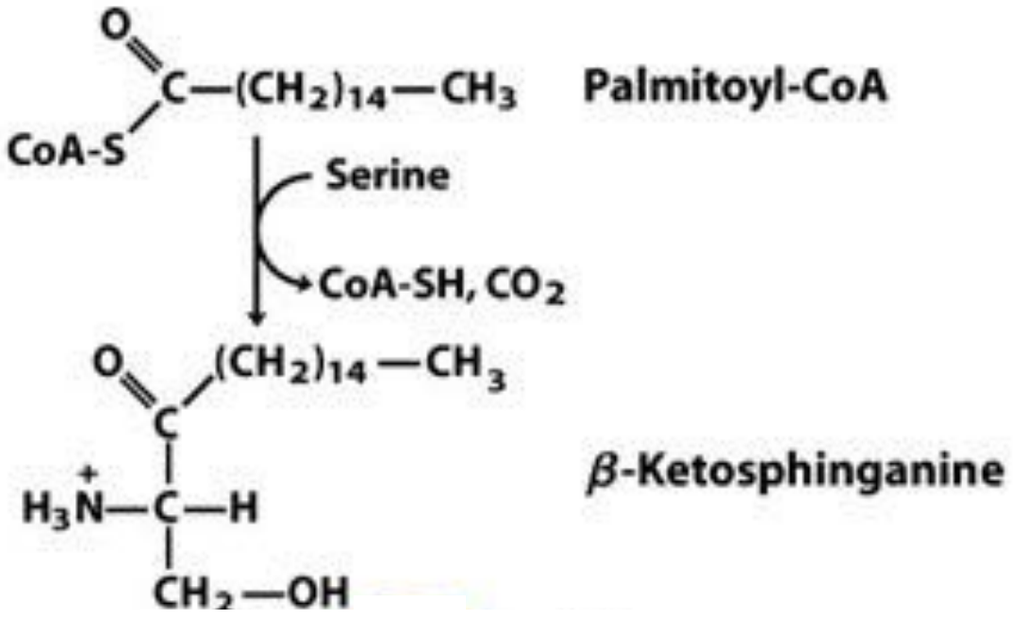


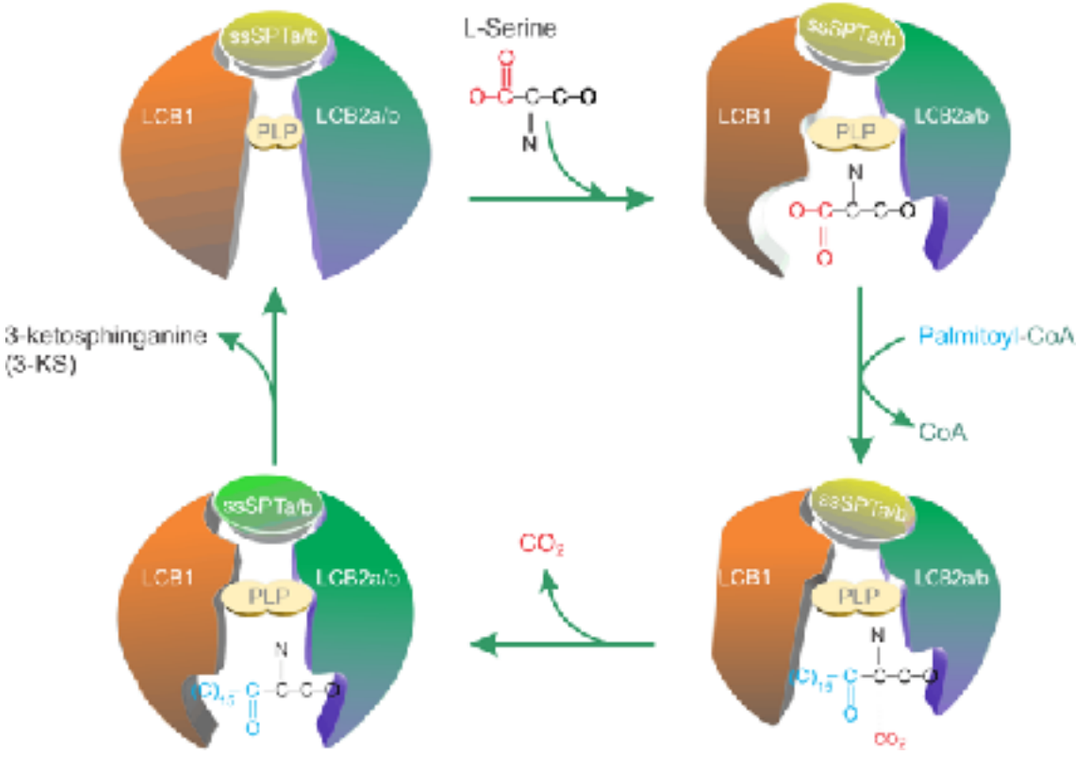
Sphingolipids can be synthesised through a *de novo* pathway initiated by the condensation of serine with an acyl-CoA molecule or through a *salvage* pathway whereby sphingoid bases (mostly sphingosine) obtained from the degradation of complex sphingolipids serve as substrates for the synthesis of new ones.

In the *de novo* pathway ceramide is obtained in 4 steps:

- Condensation
- Reduction
- Condensation
- Desaturation

Ceramide synthesis- STEP1



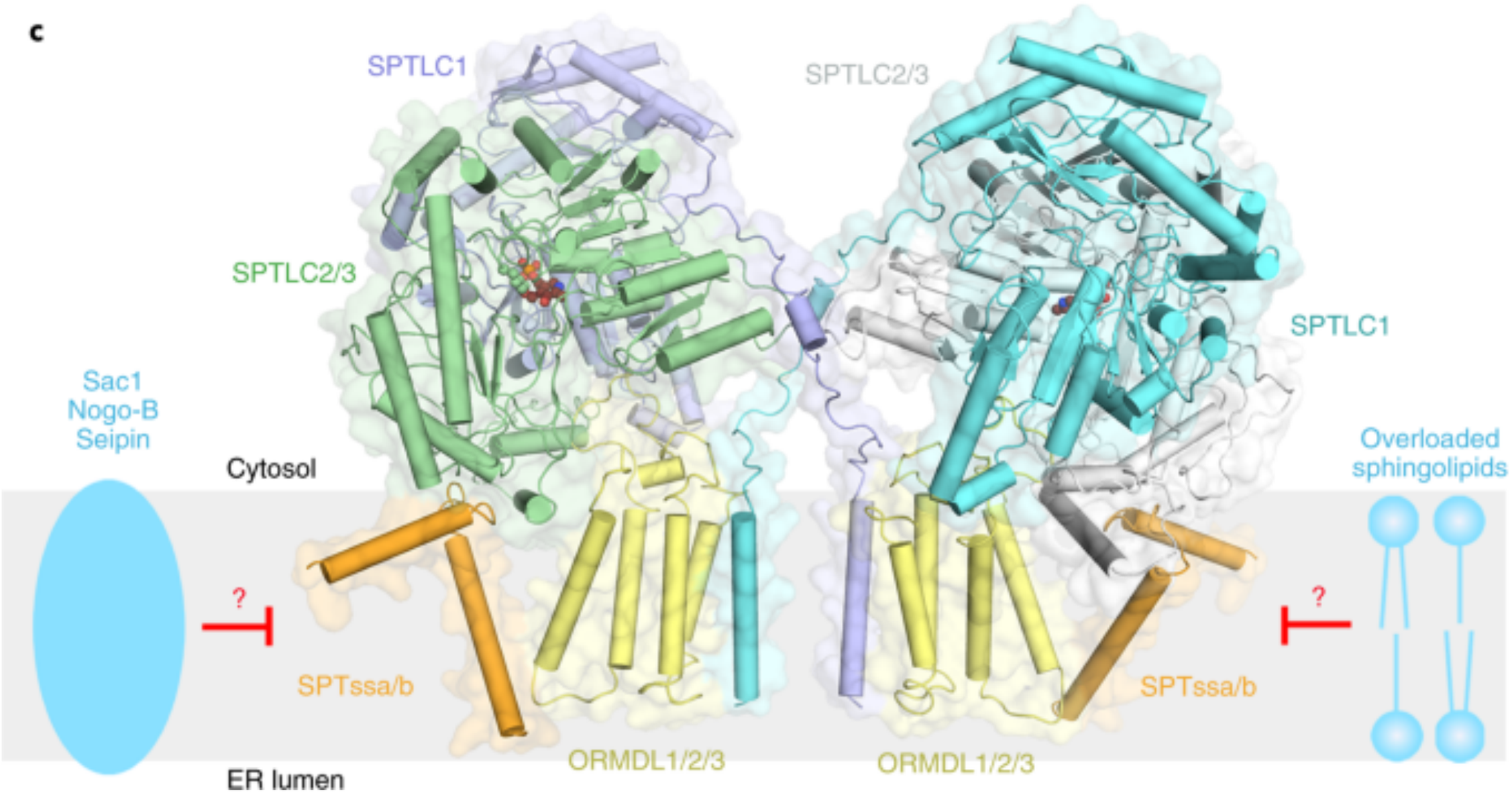


In **STEP 1:** a serine and a palmitoyl-CoA molecule are condensed by the enzyme named **serine-palmitoyltransferase** (**SPT**) to form ***β*-ketosphinganine** with the release of a CO2 molecule.

SPT has pyridoxal 5′-phosphate (PLP) as a cofactor. Initially PLP is bound to an active-site lysine. The L-serine then displaces the lysine bound to PLP, forming a serine-PLP intermediate. Subsequently, the serine-PLP intermediate attacks the incoming palmitoyl-CoA. Following decarboxylation the product *β*-ketosphingosine is released and catalytically active PLP is reformed.in

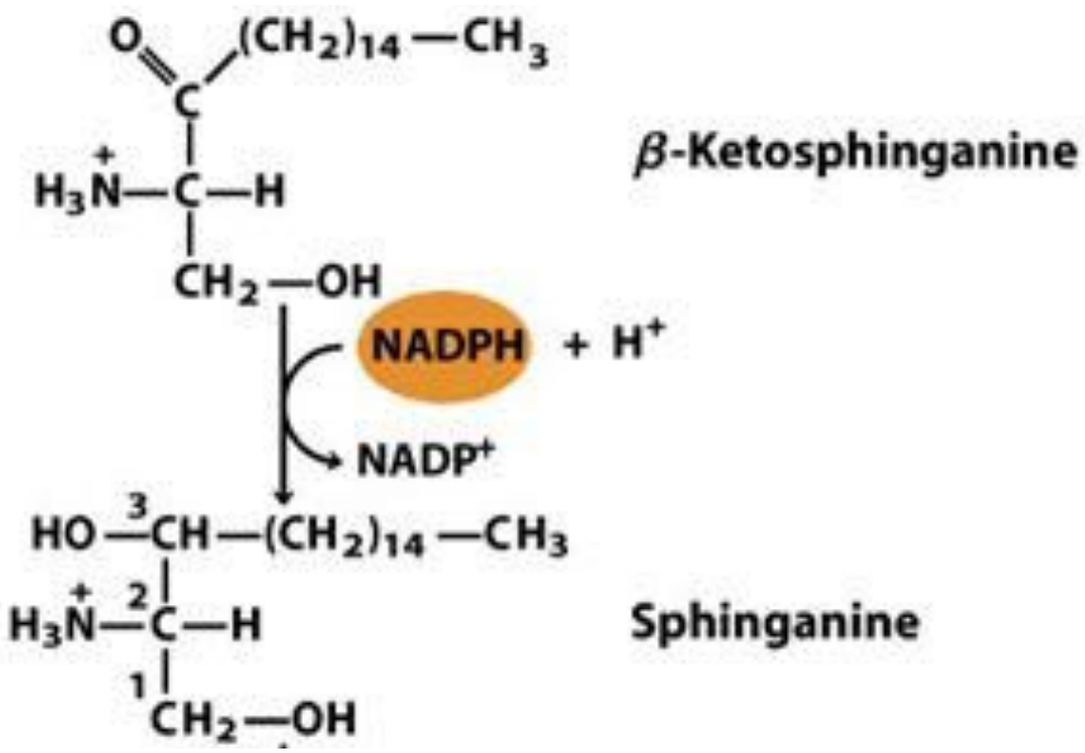
In eukaryotes, SPT is heterodimeric and localised to the **Endoplasmic Reticulum** with its active site facing the cytosol

Ceramide synthesis- STEP1



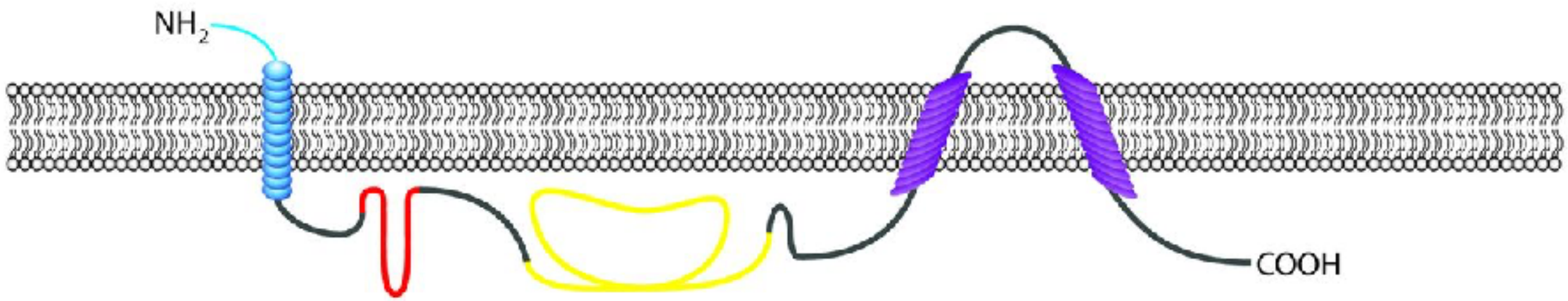
STEP 1 is homeostatic ally regulated

Ceramide synthesis- STEP2

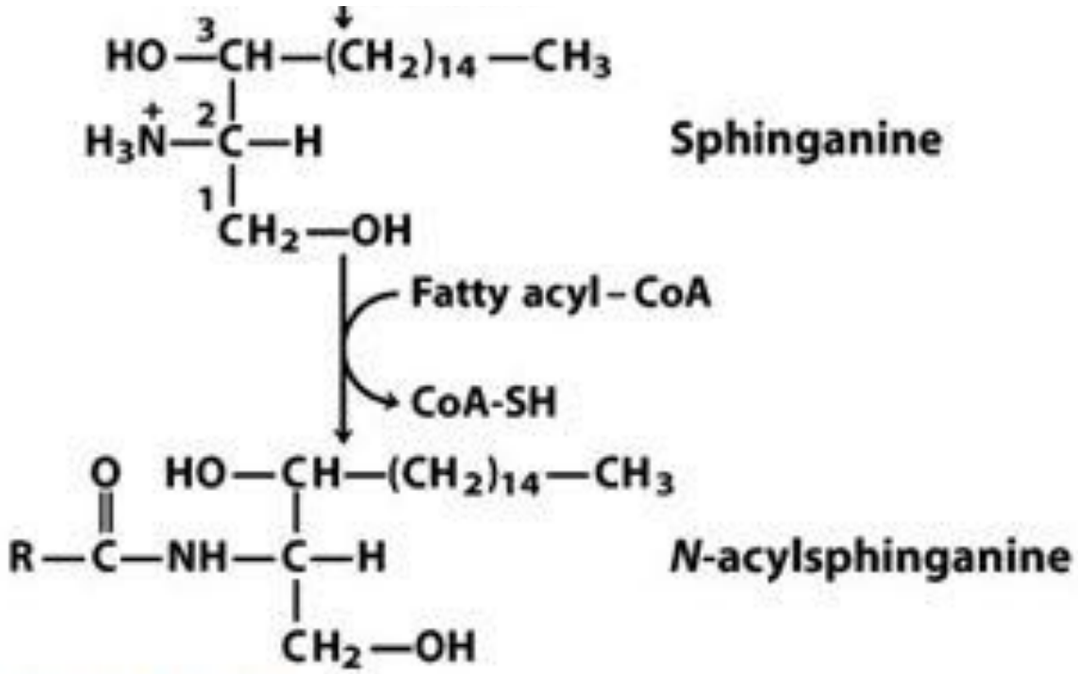


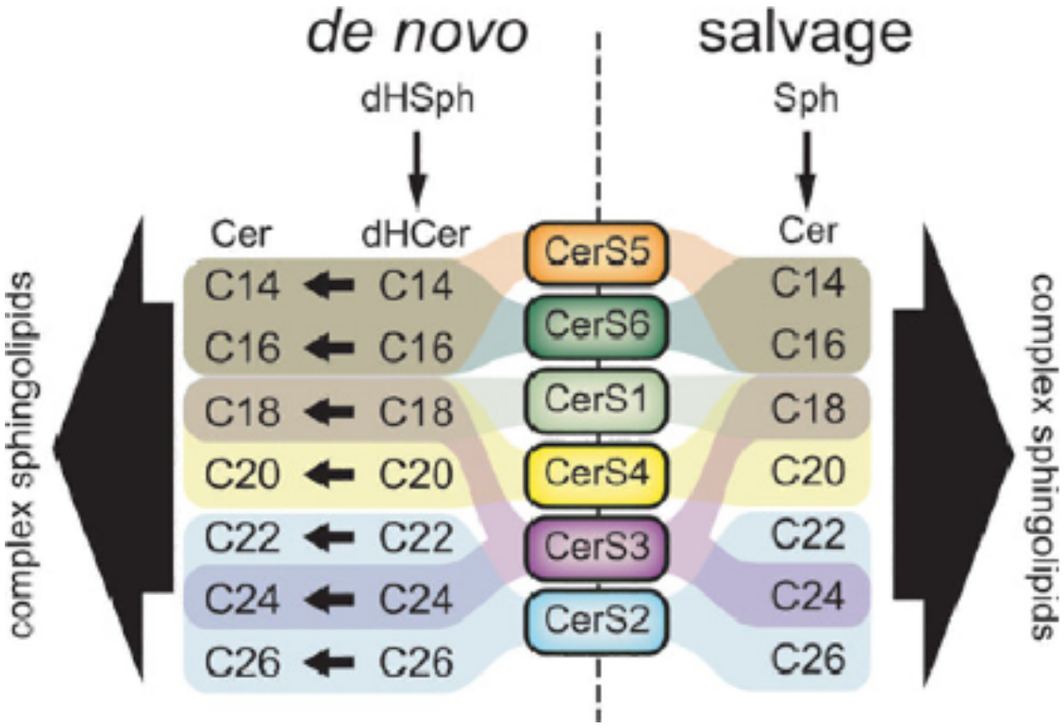
In **STEP 2:** *β*-ketosphingosine is reduced to **sphinganine** by the enzyme ***β*-ketosphinganine reductase** (**KDRS**) with consumption of an NADPH molecule.

KDSR like SPT is an integral ER protein and has its active site oriented towards the cytoplasmic leaflet of the lipid bilayer of the ER membrane.



Ceramide synthesis- STEP3



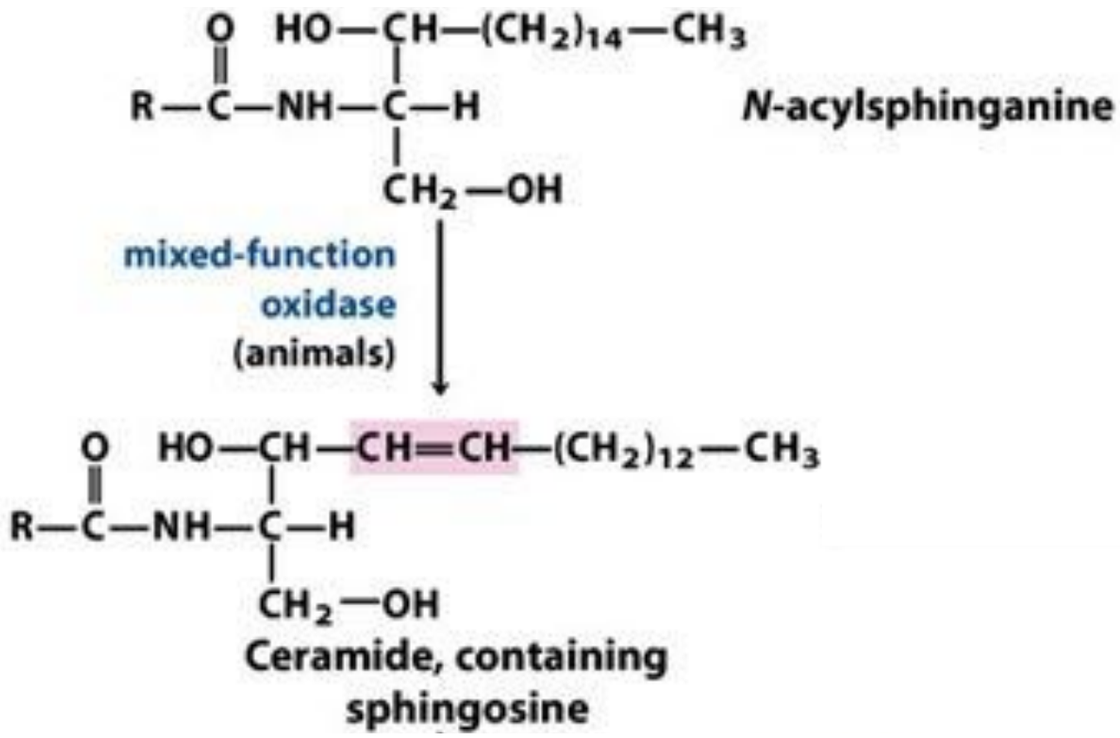


In **STEP 3:** sphinganine is condensed with a fatty acyl-CoA to produce **N-acylsphinganine** (aka **dihydroceramide**) with release of a free CoA molecule.

In mammals six enzymes named **ceramide synthases 1-6** (**CerS1-6**) catalyse this reaction. CerSes are multi-span transmembrane proteins localised to the endoplasmic reticulum.

Individual CerSes have specificity towards their acyl-CoA substrate with the three most widely expressed CerSes (i.e., CerS2, CerS4, and CerS5) having preference for C22-C24-C26, C-20, and C14-C16-C18 acyl chain respectively.

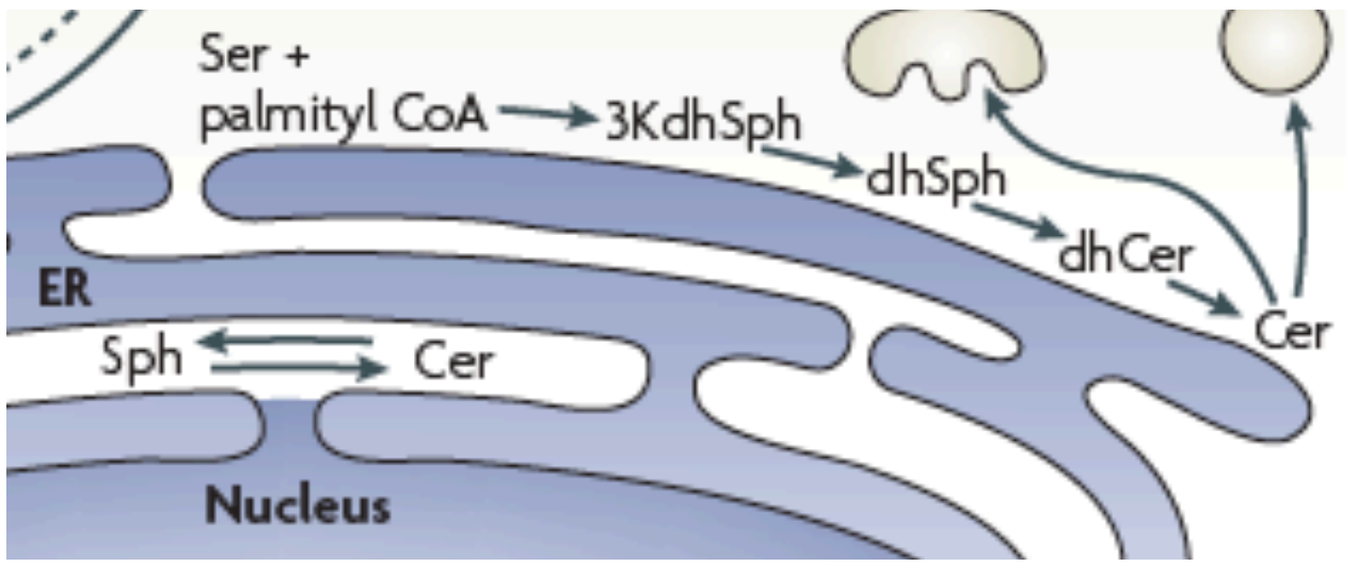
Ceramide synthesis- STEP4



In **STEP 4:** N-acylsphinganine (dihydroceramide) is *desaturated* to **ceramide** by the enzyme **dihydroceramide desaturase** (**DES**).

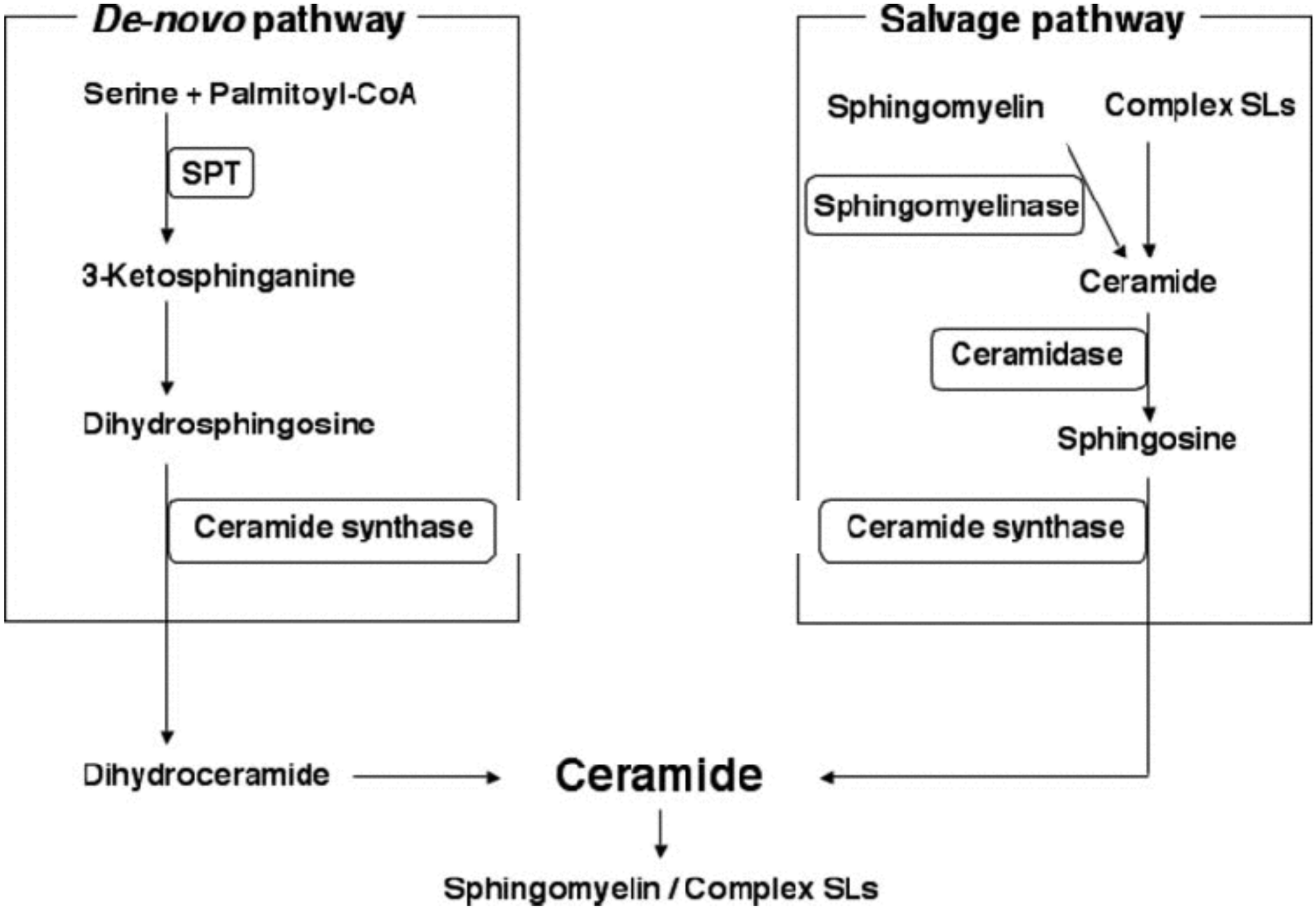
DES inserts a 4,5-*trans*-double bond to the sphingolipid backbone of the dihydroceramide. DES requires the O2 and NADPH as cofactors.

Like SPT, KDSR, and CerS1-6, DES is an integral ER protein.

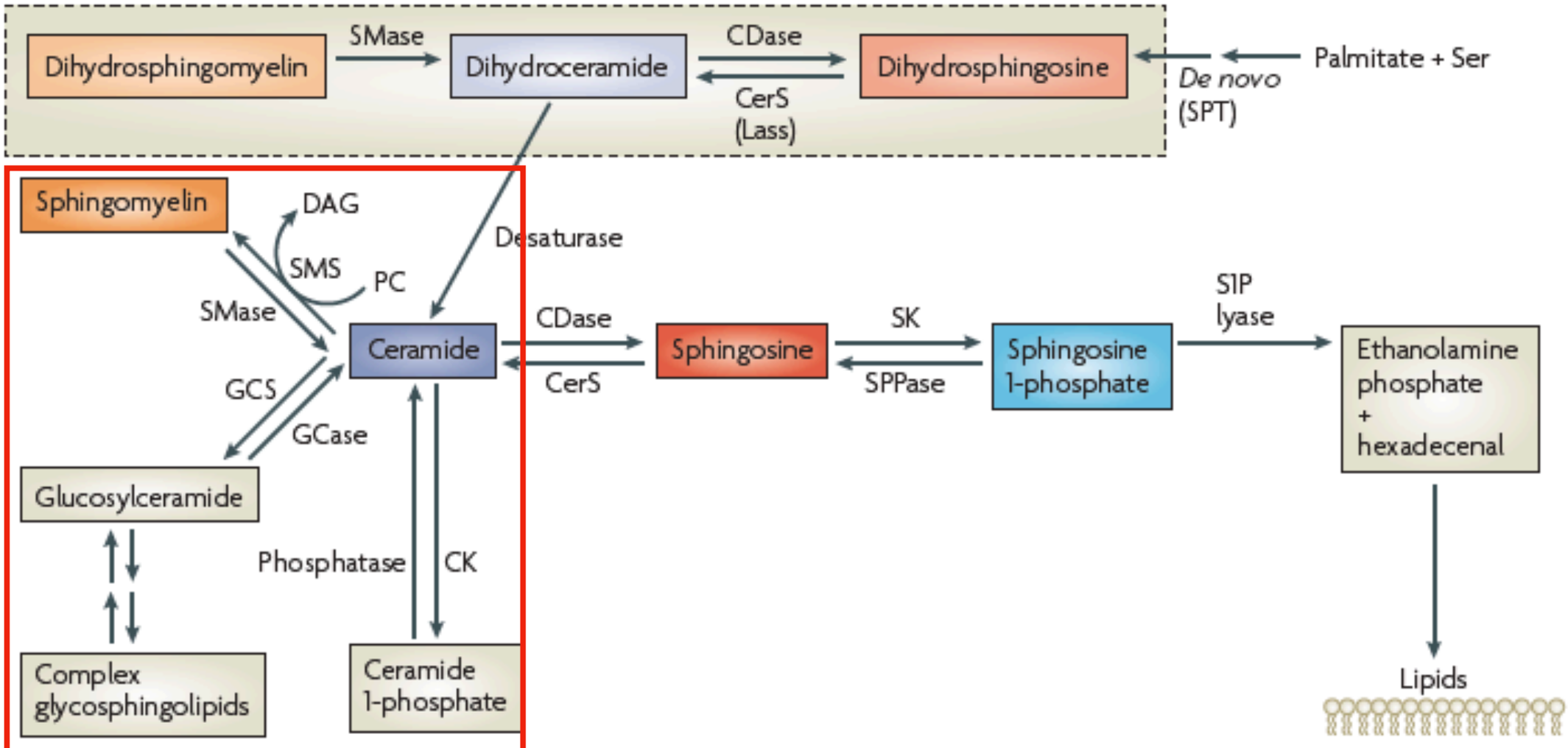


The overall de novo Cer synthesis takes place in the Endoplasmic Reticulum with the hydrophobic intermediates of these reaction facing the cytosolic environment

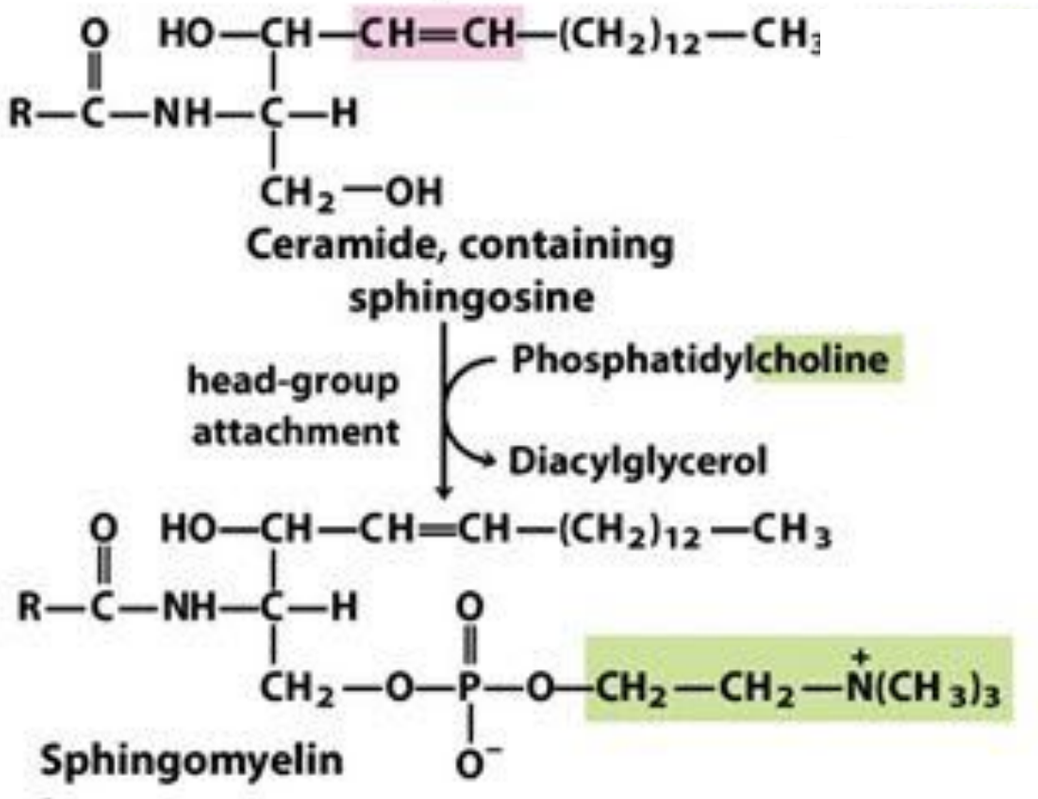
The salvage pathway



Ceramide processing

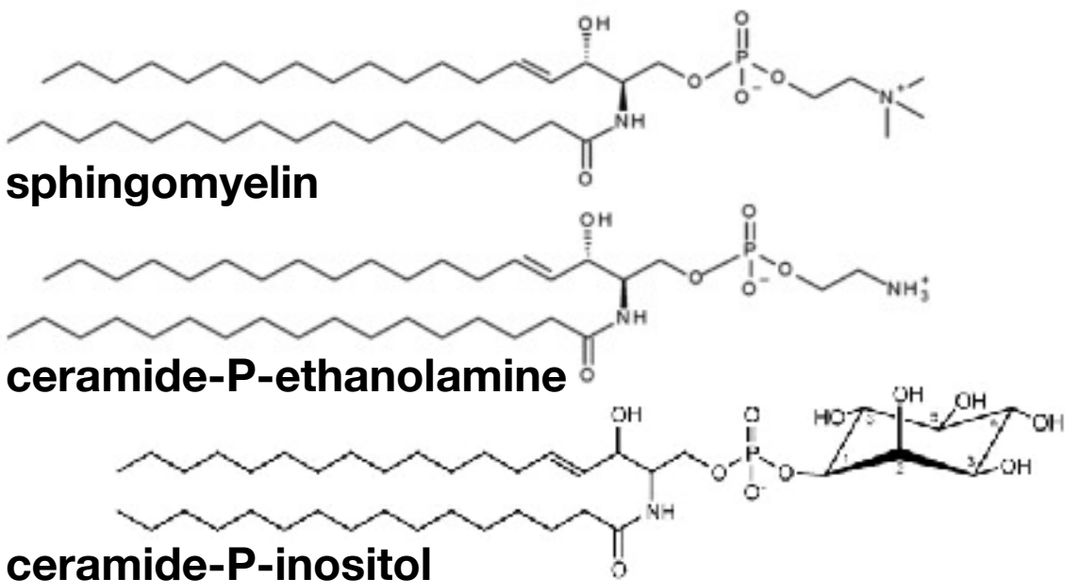


Sphingomyelin Synthesis



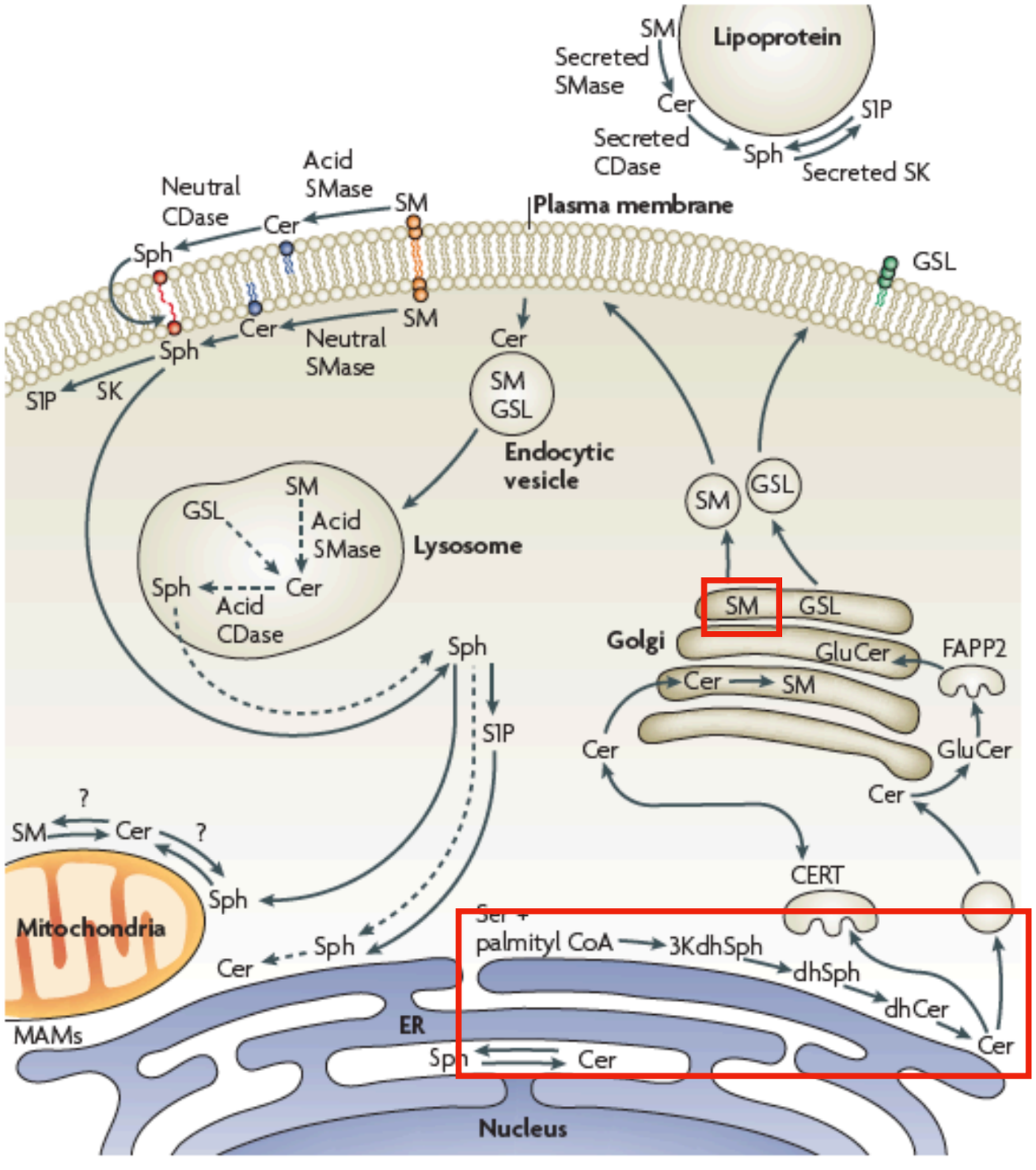
Once formed ceramide can be converted into **sphingomyelin** by the enzyme **sphingomyelin synthase** (**SMS**).

SMS is multispan integral membrane protein that catalyses the transfer of a phosphocholine head group from phosphatidylcholine to ceramide yielding thee production of **sphingomyelin** (i.e., phosphocoline-ceramide) and **diacylglycerol**.



While in vertebrates the major phoposphingolipid species is sphingomyelin (SM) in invertebrates, plants and yeasts ceramide-phospho ethanolamine (CPE) and inostol-phospho ceramide (IPC) predominate. Their synthesis is accomplished through and enzymatic mechanism very similar to that catalysed by SMS.

Sphingomyelin Synthesis



In vertebrates ceramide is produced in the cytosolic leaflet of the ER membrane bilayer while sphingomyelin is synthesised in the lumen of the *trans* Golgi compartment.

This poses a topological problem on how the SMS substrate (i.e., ceramide) can be presented to the active site of SMS.

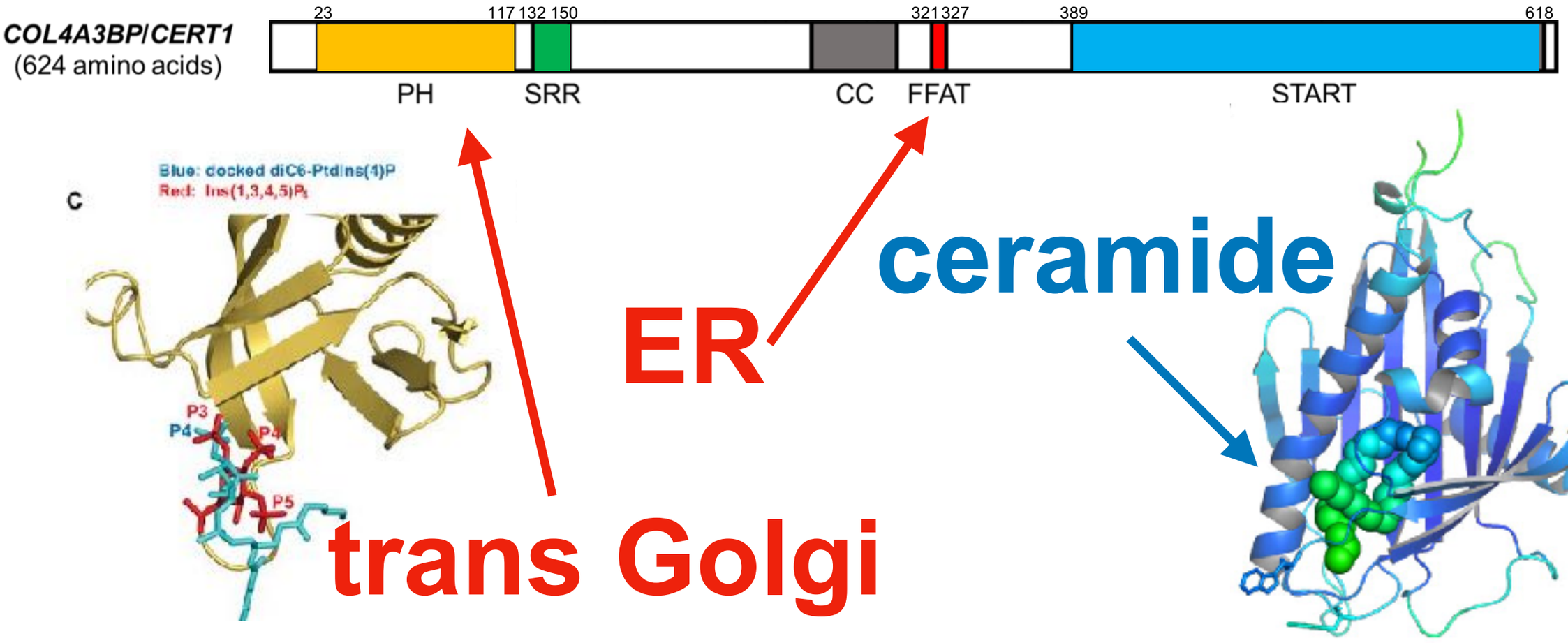
While trans-bilayer movement happens spontaneously for ceramide, its diffusion through the aqueous cytosolic environment is highly unfavourable.

Thus ceramide needs to be actively transoprted from the ER to the *trans* Golgi compartment.

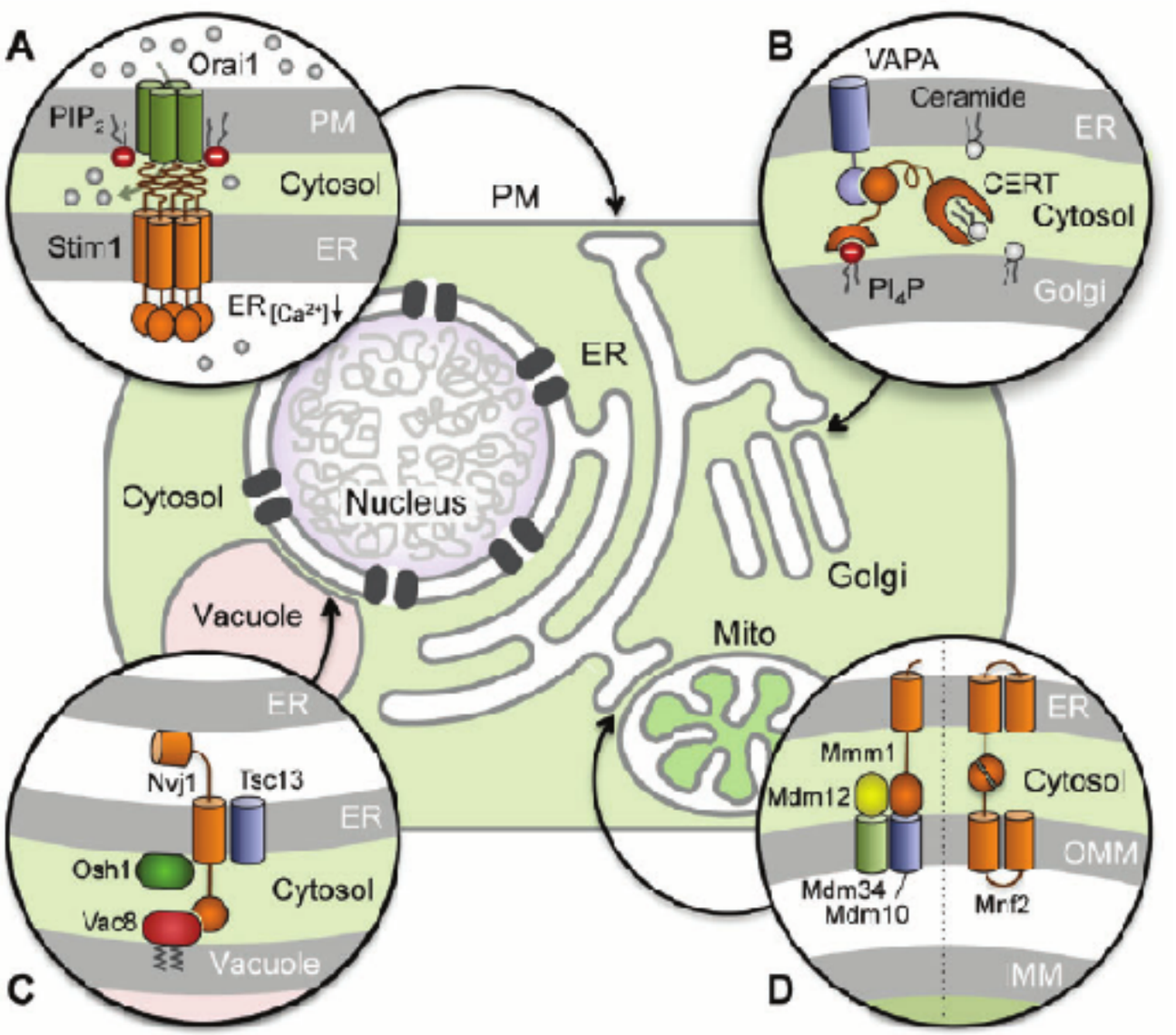
Sphingomyelin Synthesis- CERT

Ceramide is transported from the ER to the trans Golgi by the cytosolic lipid transfer protein **CERT1**.

CERT1 is a multi domain protein with an N-terminal plekstrin homology (PH) domain that recognises the trans Golgi membranes [via binding the phosphoinositide PtdIns(4)*P*] and FFAT motif that interacts with the integral ER protein VAP and a C-terminal START domain that can extract ceramide from the membrane bilayer and transport through the cytosol.

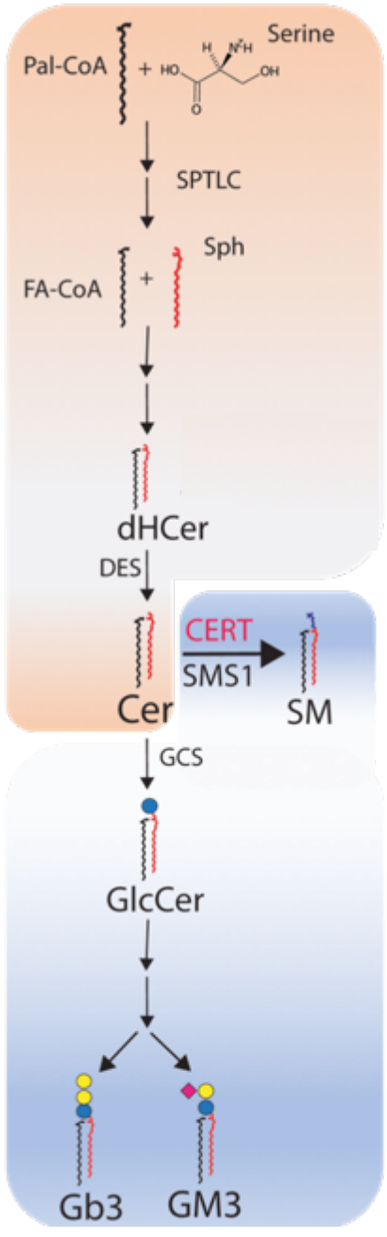


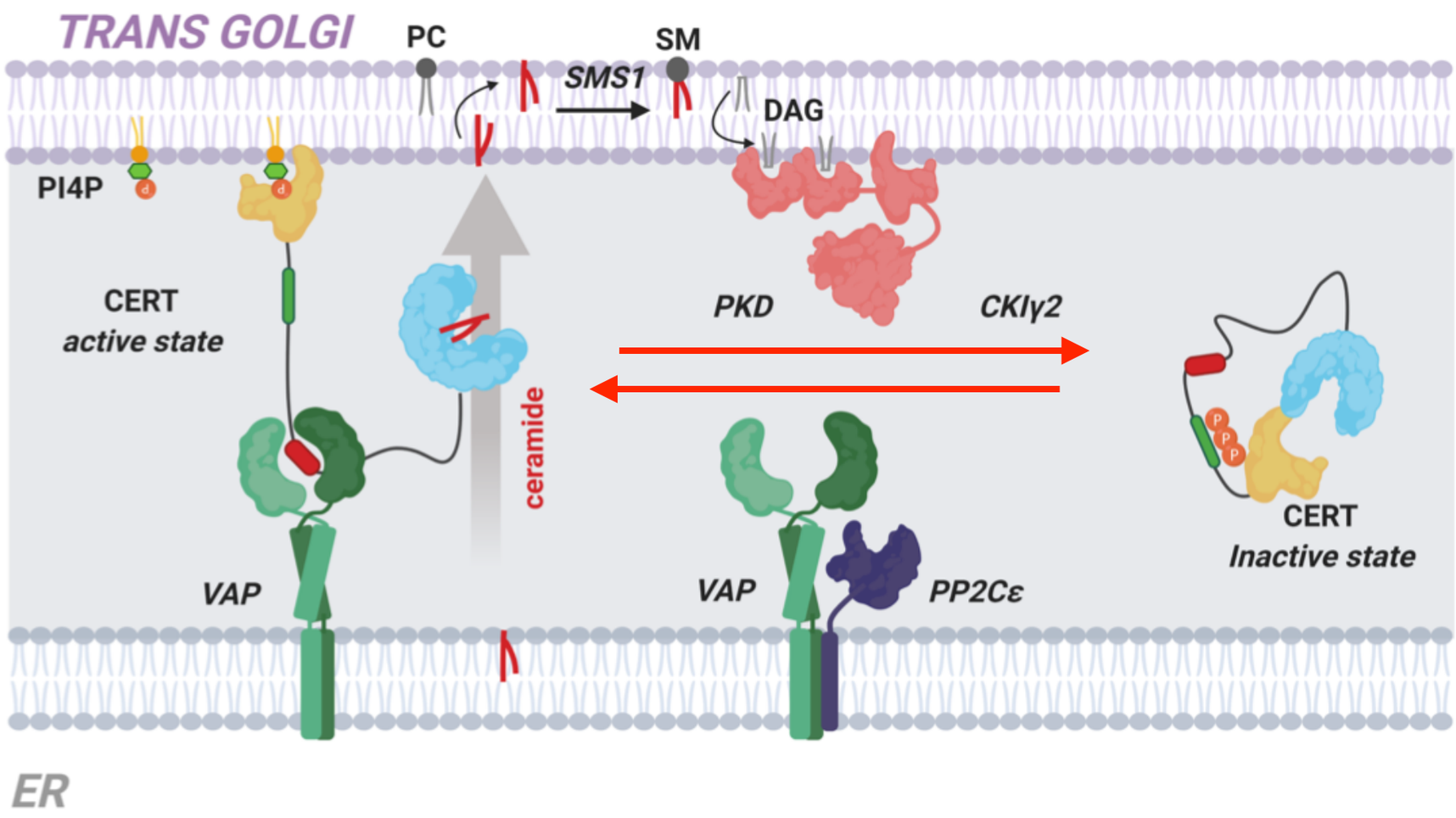
Sphingomyelin Synthesis- CERT



Membrane contact sites (**MCS**) are close appositions between two organelles with an intermembrane distance in the order of the size of a single protein, 10-15 nm. The ER makes close contact with many organelles, including mitochondria, Golgi, endosomes, lysosomes, peroxisomes, chloroplasts and the plasma membrane. CERT1 operates ceramide transfer at ER-trans Golgi- MCS.

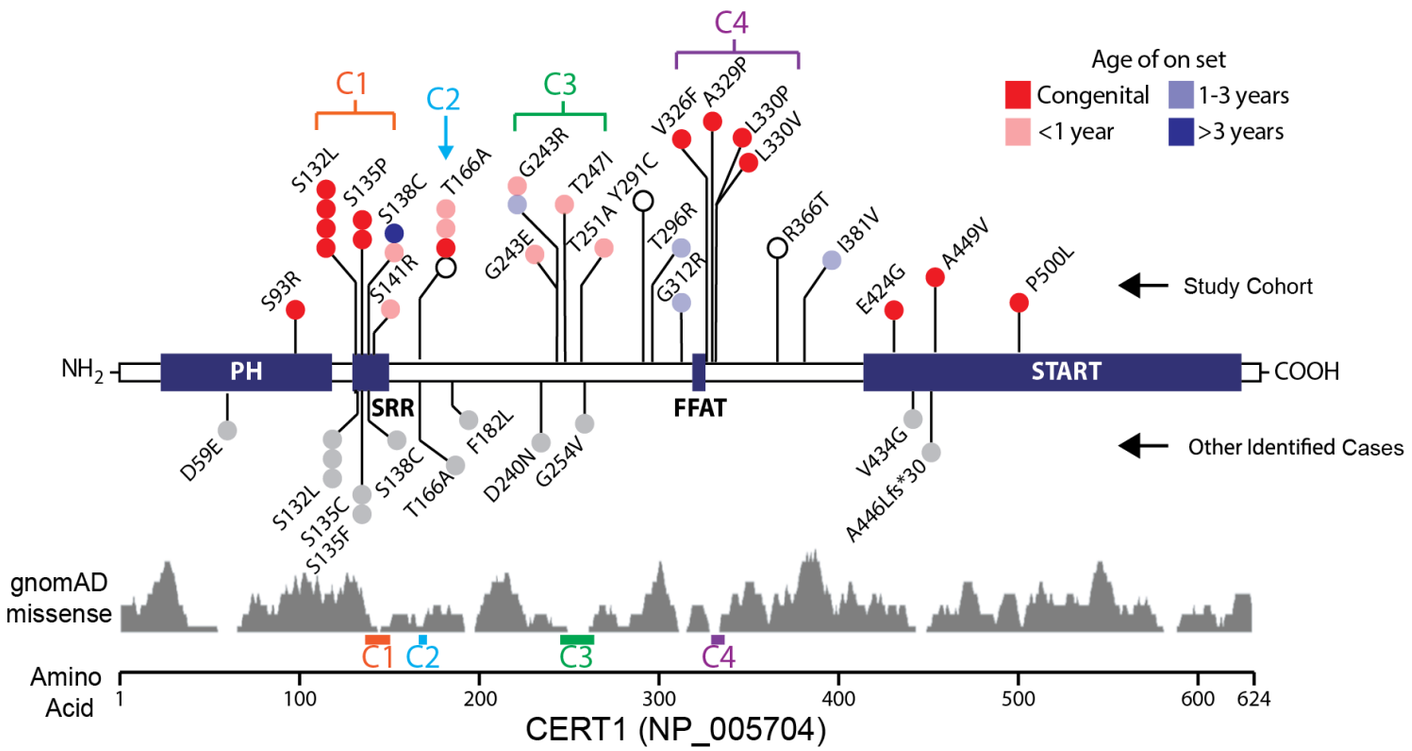
Sphingomyelin Synthesis- CERT

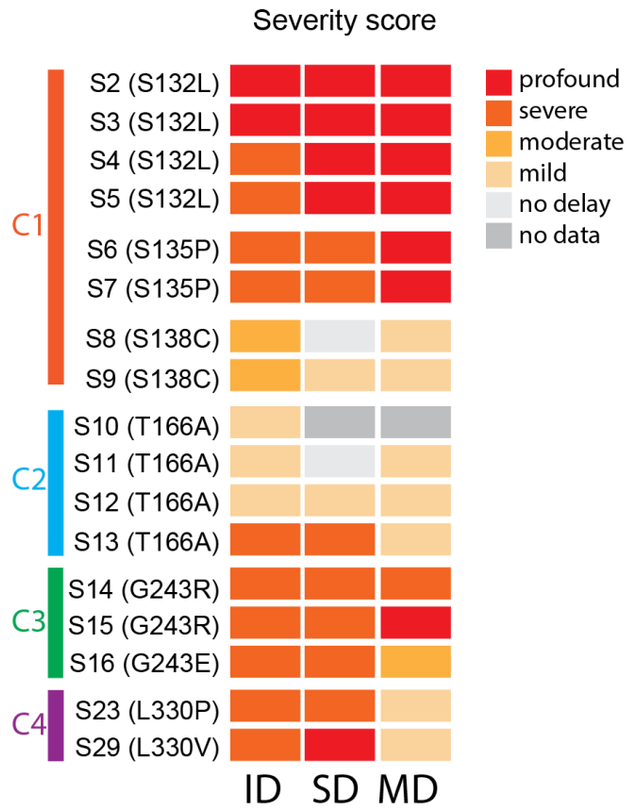


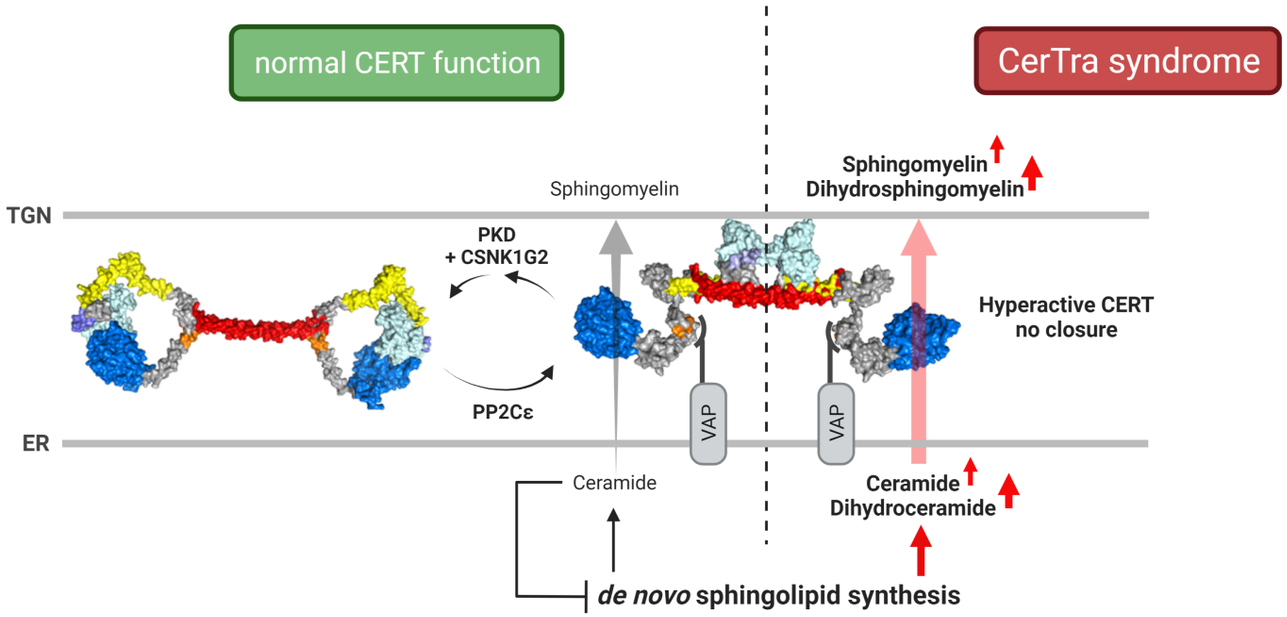


CERT1 is a regulated factor in sphingolipid metabolism. Indeed, ceramide transport from the ER to the Trans Golgi is tightly controlled through an homeostatic mechanism based on CERT inactivation upon excessive sphingomyelin production.

Sphingomyelin Synthesis- CERT

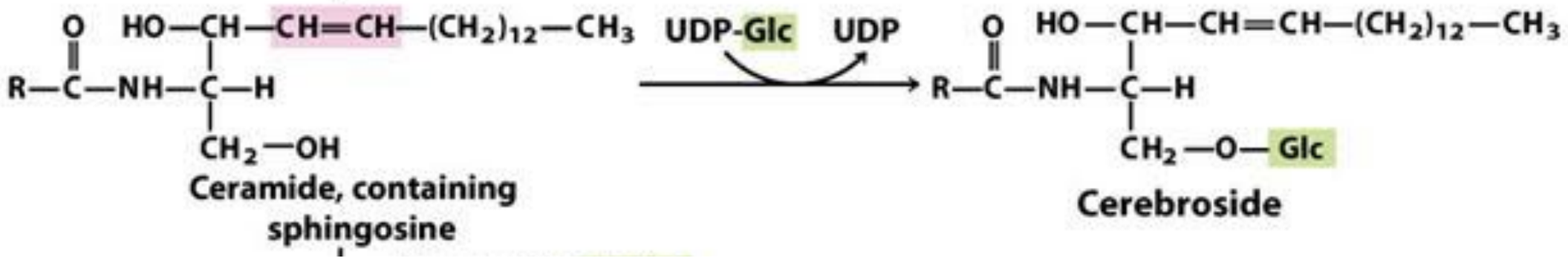


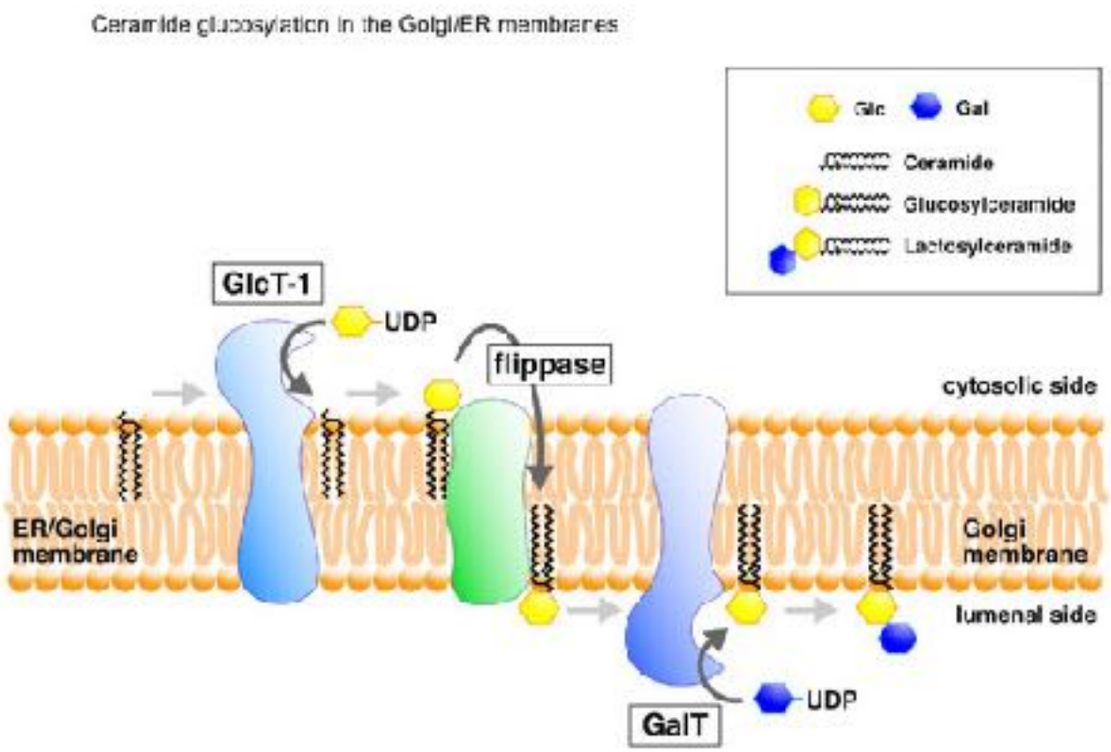




Mutations in CERT1 are associated with intellectual disability. Several variants fall into regulatory protein domains or into a previously uncharacterized dimeric helical domain that enables CERT homeostatic inactivation, without which sphingolipid production goes unchecked.

Glucosylceramide Synthesis

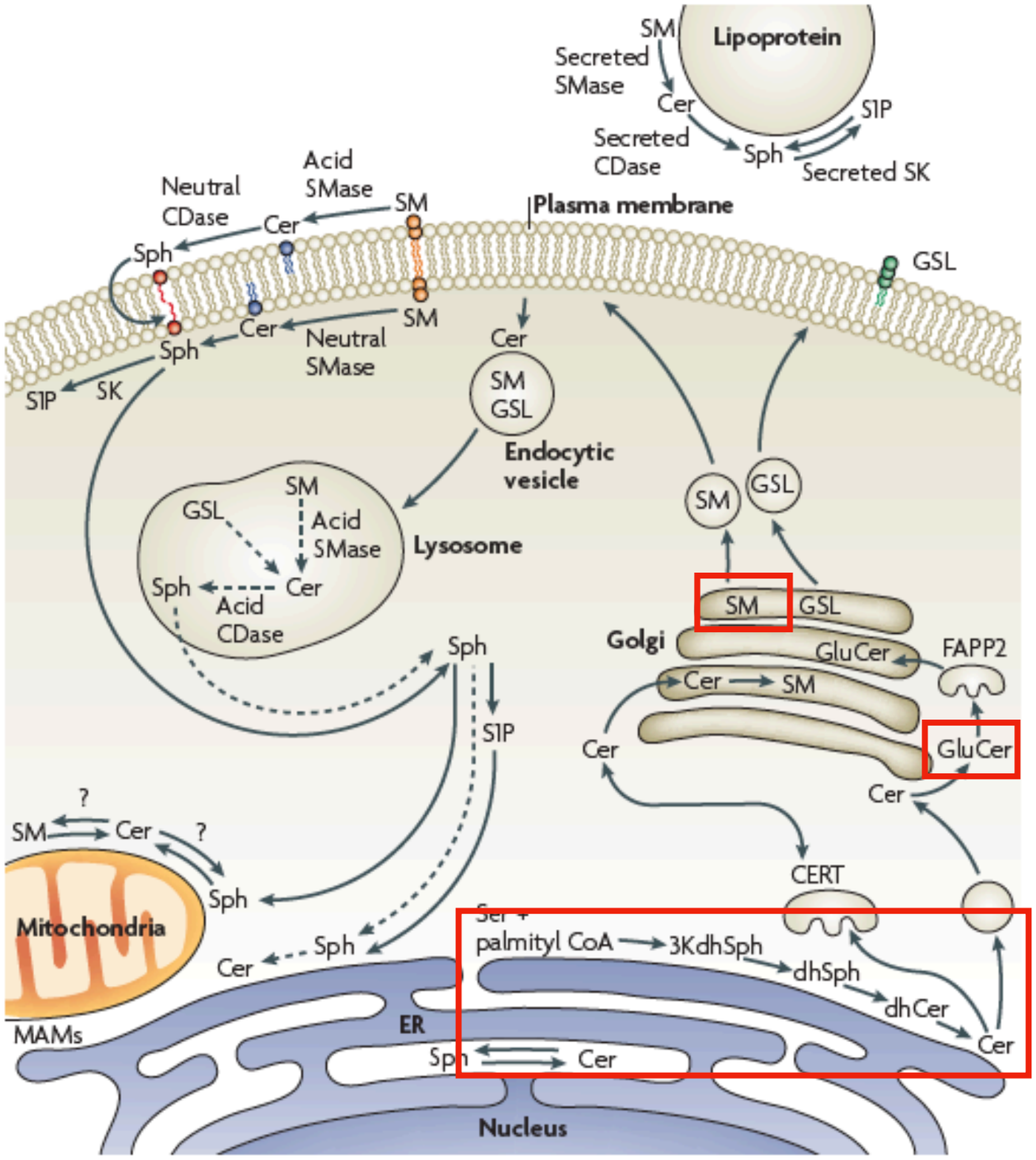




An alternative fate of newly formed ceramide is to be converted into **cerebroside** (**glucosylceramide, GlcCer**) by the enzyme **GlcCer synthase** (**GCS**).

Like SMS, GCS is multispan integral membrane protein. GCS catalyzes at the cytosolic surface of the Golgi, the initial step of the glucosylceramide-based glycosphingolipid/GSL synthetic pathway, the transfer of glucose from UDP-glucose to ceramide to produce glucosylceramide/ GlcCer.

Glucosylceramide Synthesis

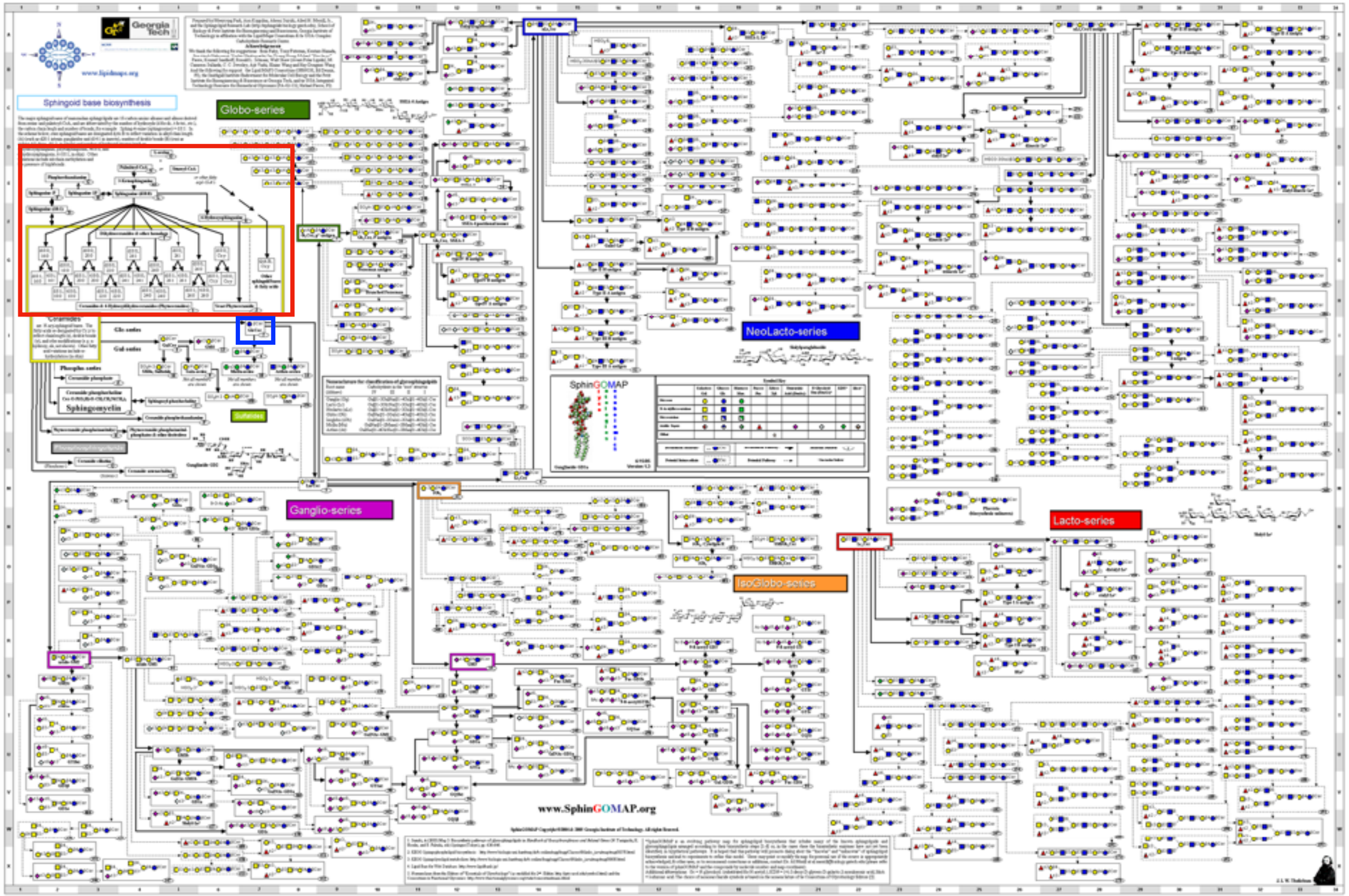


While sphingomyelin is produced in the lumen of the *trans*-Golgi, GlcCer is synthesised on the cytosolic leaflet of the cis-Golgi membranes.

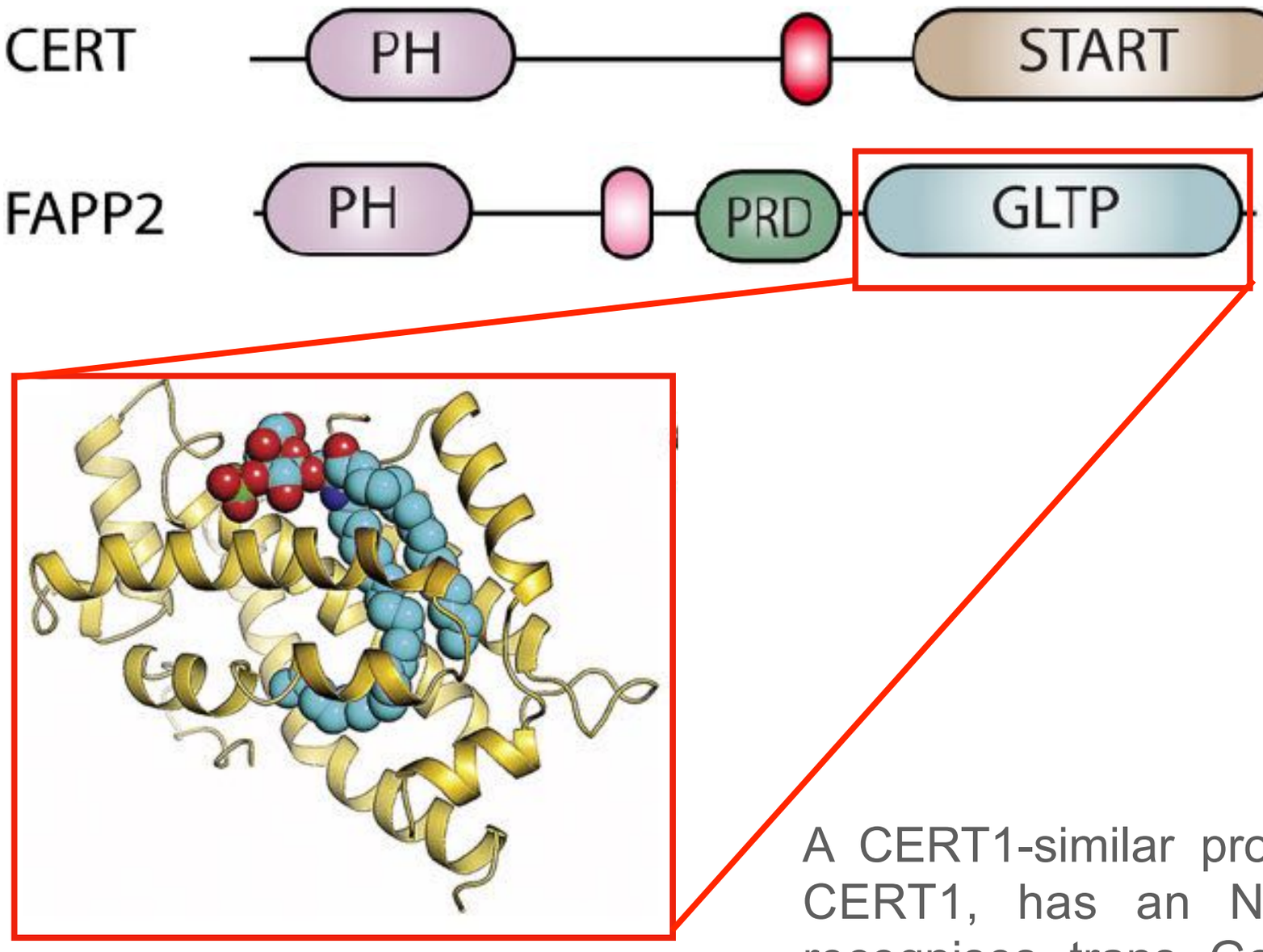
Thus while vesicles derived from the ER will convey ceramide to the cis Golgi for GlcCer synthesis, CERT1, by delivering ceramide directly to the trans Golgi commits it to sphingomyelin production.

After its production, GlcCer is transported to the trans Golgi compartment where, after translocation to the lumens membrane aspects is further processed to complex glycosphingolipids

Glycosphingolipid Synthesis

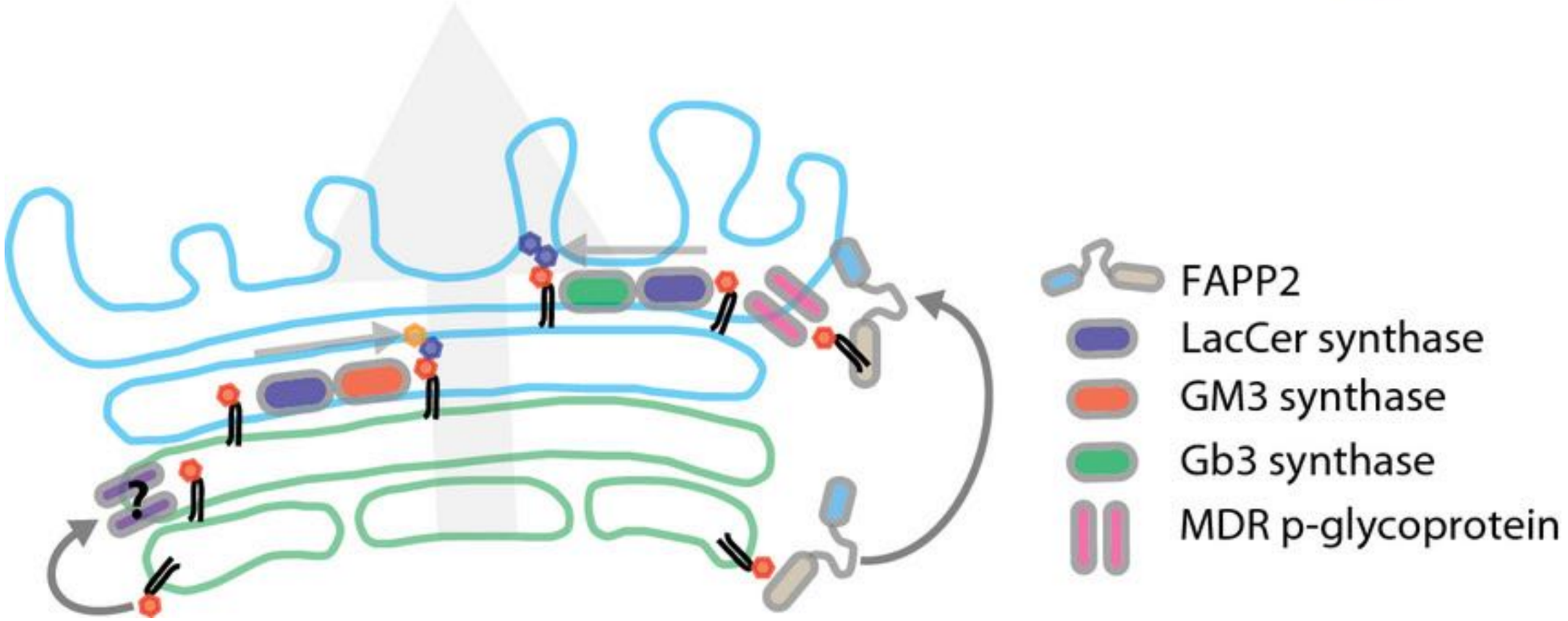


Glycosphingolipid Synthesis



A CERT1-similar protein is FAPP2. FAPP2, like CERT1, has an N-terminal PH domain that recognises trans Golgi membranes via binding PtdIns(4)*P* and a C-terminal (glyco)lipid transfer protein (GLTP) domain that in the case of FAPP2 binds and transfers GlcCer

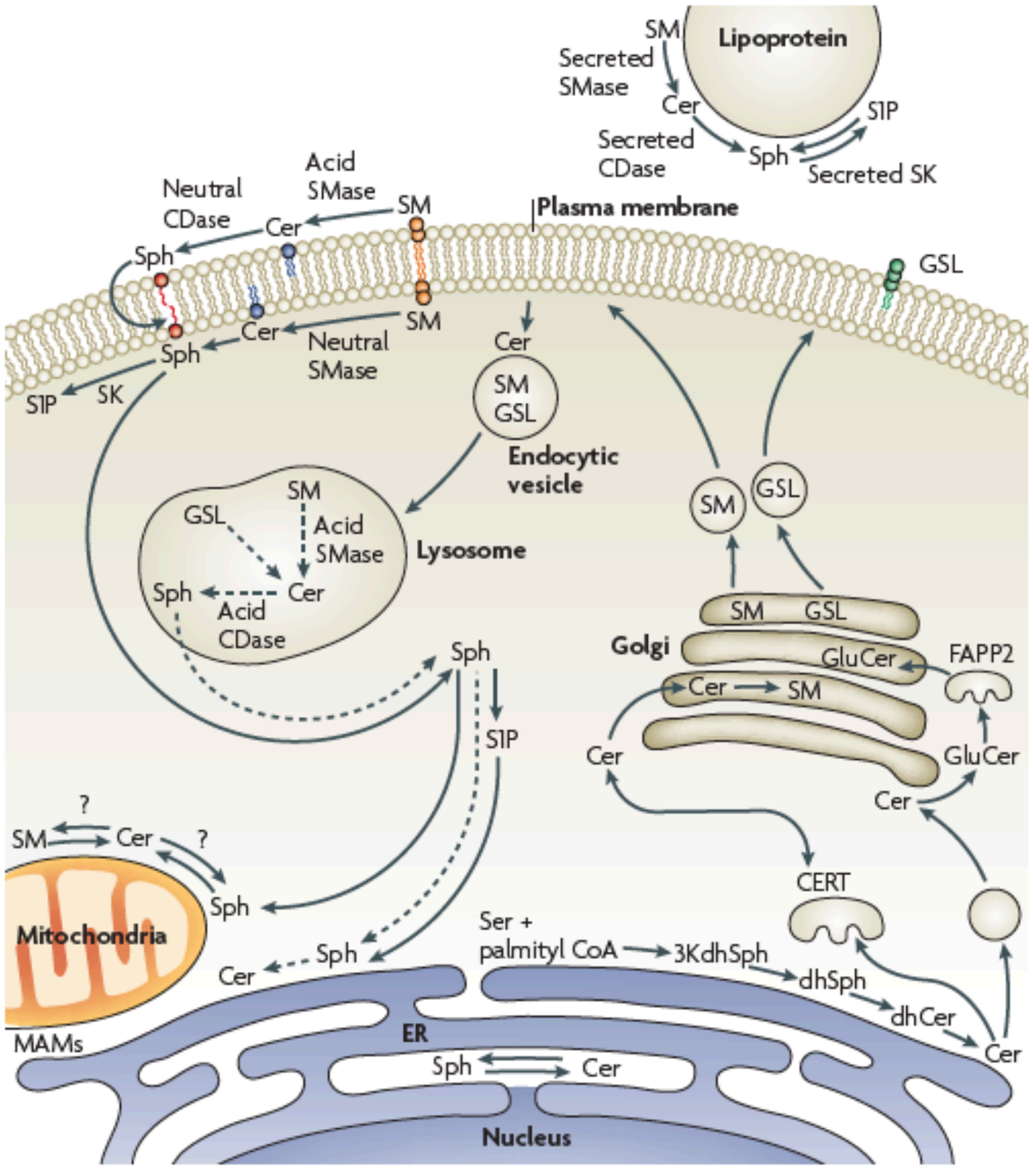
Glycosphingolipid Synthesis



FAPP2 extracts GlcCer for *cis*-Golgi membranes and delivers it to the *trans*-Golgi [skipping intermediate compartments]. At the *trans*-Golgi GlcCer is used to produce Globo series glycosphingolipids. GlcCer can also progress through the Golgi independently of FAPP2 in which case it is used to produce ganglio series glycosphingolipids.

Thus, CERT1 and FAPP2 determine the metabolic fates of their interacting lipids (i.e., ceramide and GlcCer), by subtracting them from the anteerogradee membrane flux.

Sphingolipids



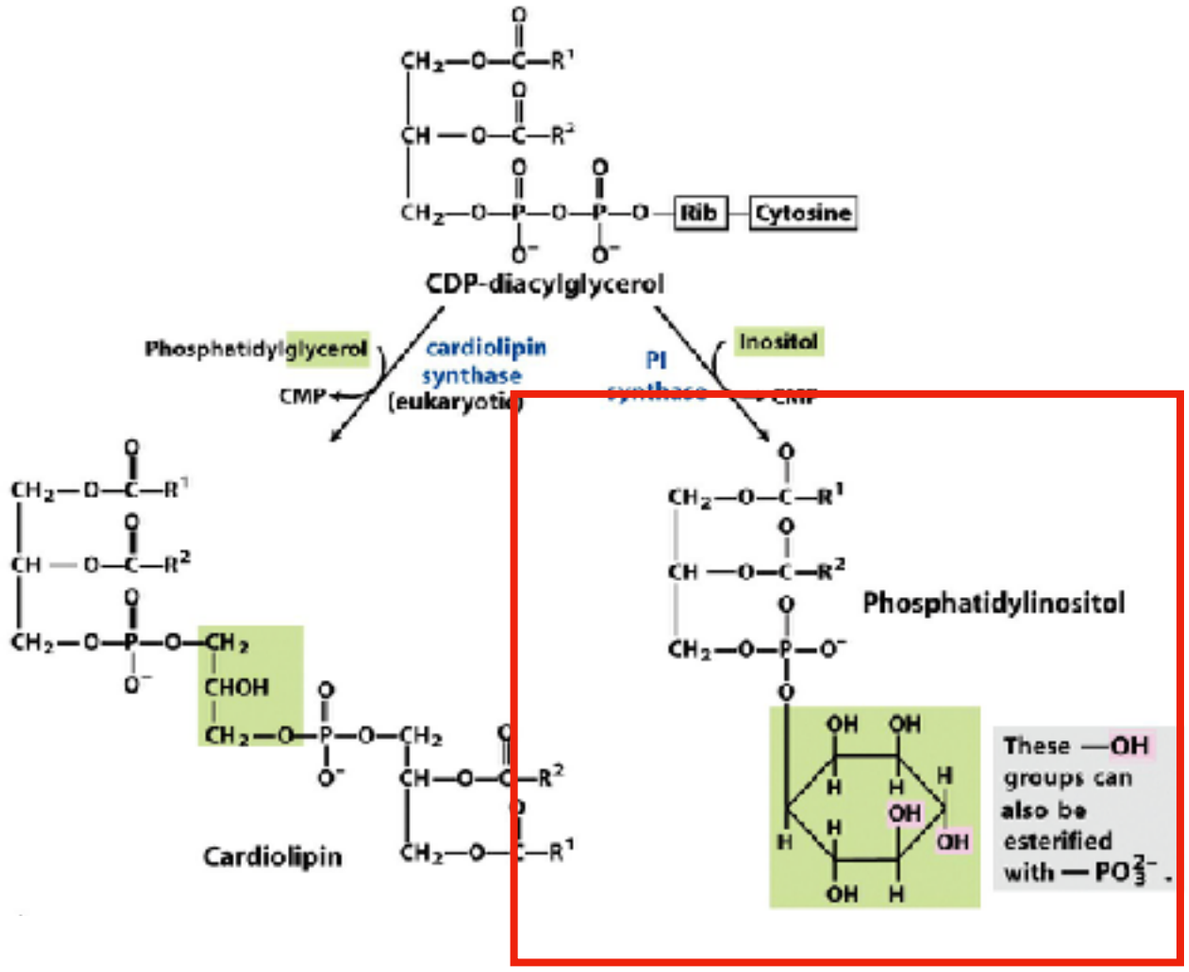
Sphingolipid synthesis is initiated on the cytosolic aspects of the ER. It can involve *de novo* synthesis (initiated by thee condensation of serine and pamitoyl-CoA) or the savage pathway where sphingosine derived from sphingolipid turnover is used to produce ceramide.

Once produced, ceramide is transported to the Golgi either non-vesicularly by CERT1 for the production of sphingomyelin of through vesicles for the production of GlcCer.

GlcCer is then translocated to the lunes of the medial Golgi to initiate ganglioside production or shunted to the trans-Golgi by FAPP2 to initiate globoside production

Break

Phosphoinositides

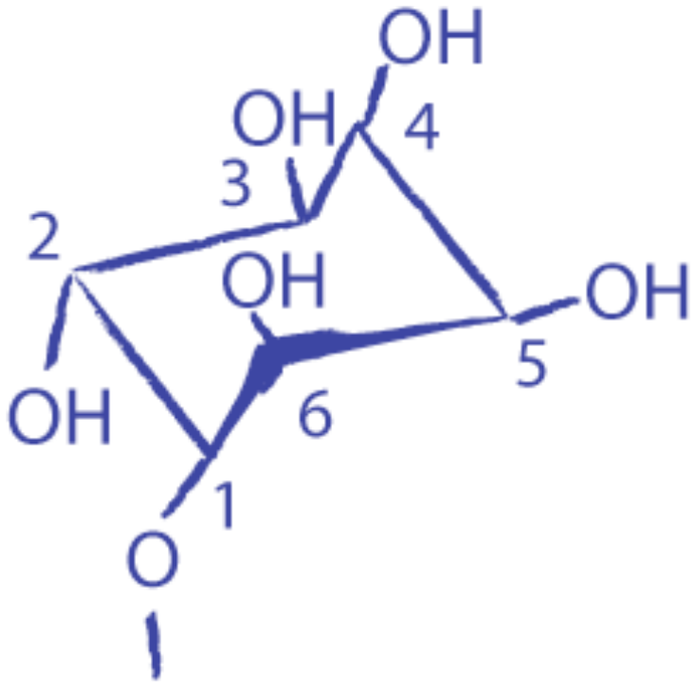


phosphatidylinositol (PtdIns), which is synthesised by the enzyme phopshatidylinositol synthase (PIS), is the precursor of a class of lipids named phosphoinositides.

Phosphoinositides are phosphorylated forms of PtdIns and play important roles in cell signaling and membrane trafficking.

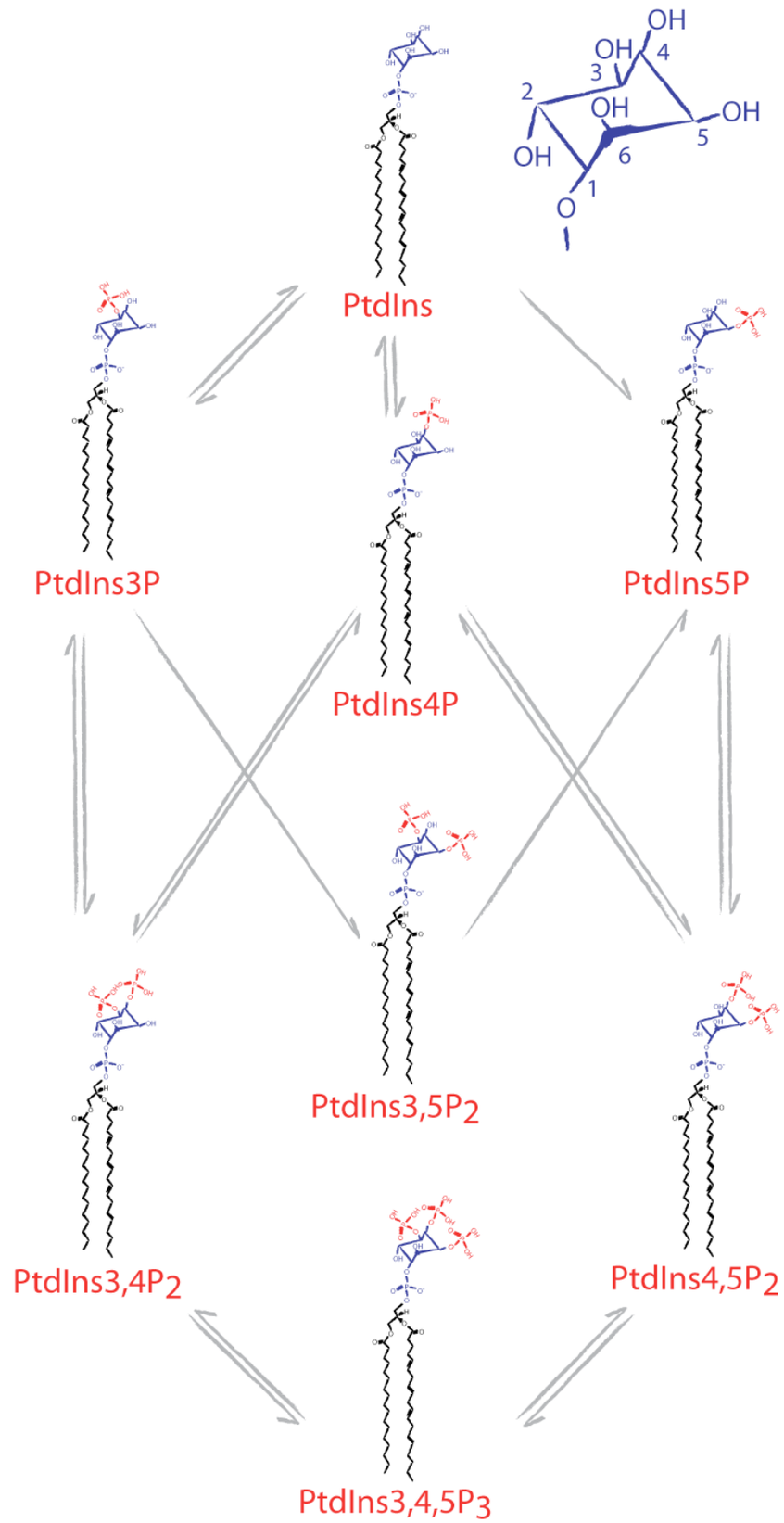
Despite their low abundance, phosphoinositides and their metabolism are crucial to the precise spatiotemporal regulation of key cellular events, where phosphoinositides can serve as **second messengers** or **modulate recruitment and/or activity of membrane proteins**

Phosphoinositides

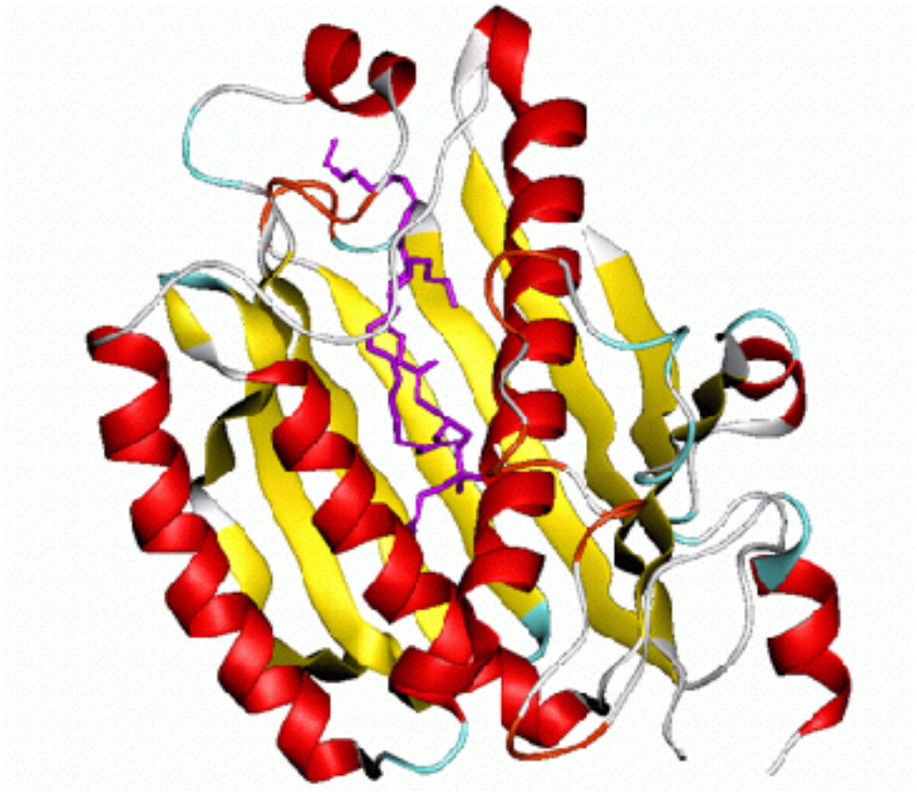


The inositol ring in phosphoinositides can be phosphorylated by a variety of kinases on the three, four and five hydroxyl groups in seven different combinations. However, the two and six hydroxyl groups are typically not phosphorylated due to steric hindrance.

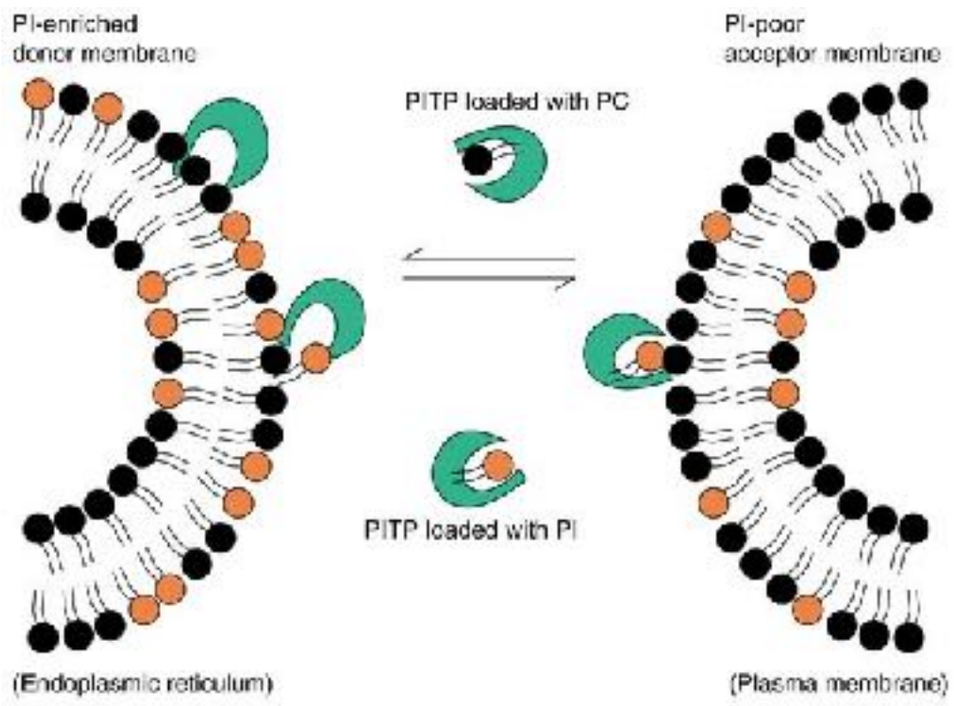
While PtdIns is synthesised in the ER its processing takes place in a variety of sub cellular compartments. Thus PtdIns needs to be transported across thee cytosol.



PITPs

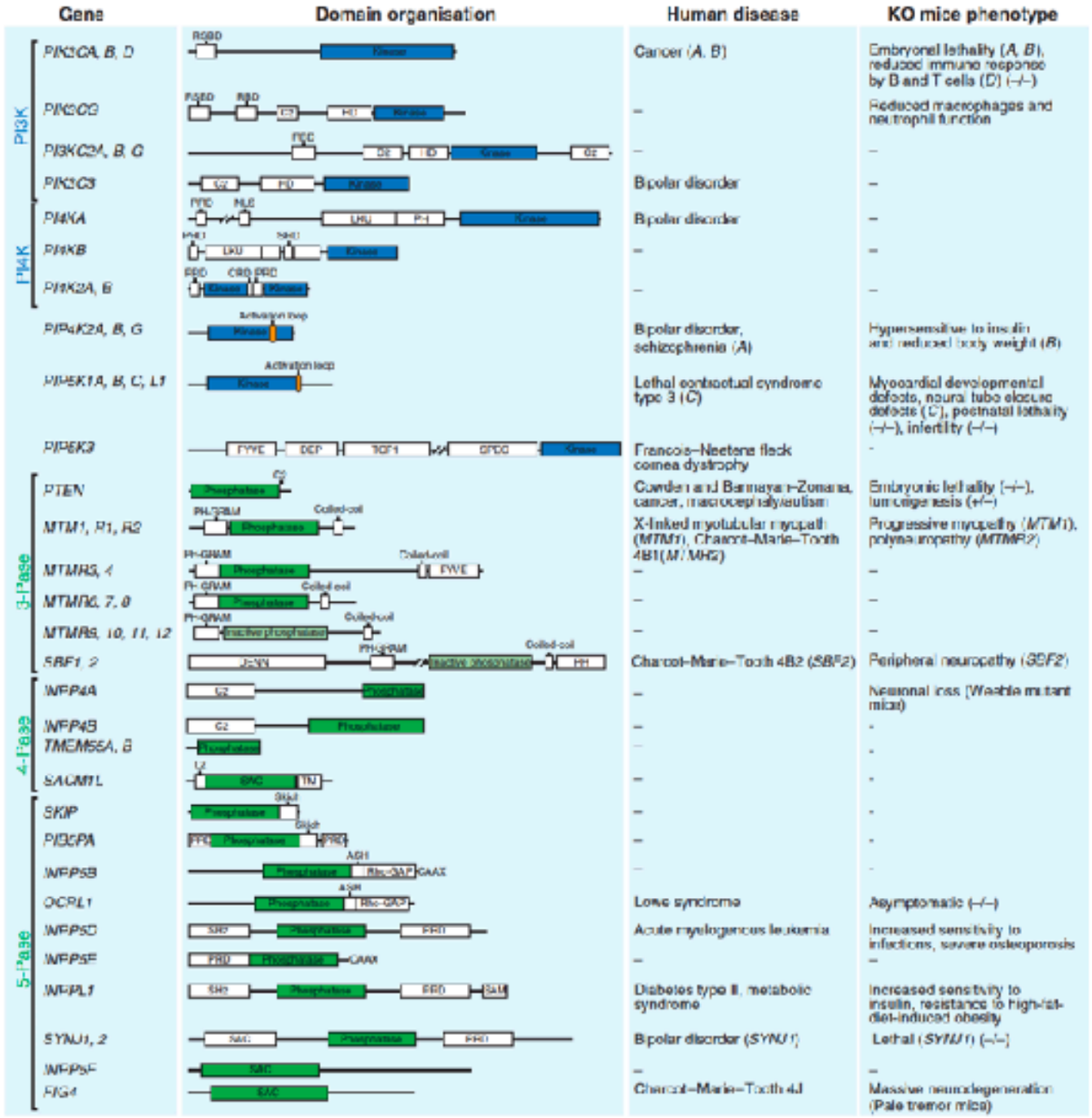


phosphatidylinositol transfer proteins (PITPs) are phospholipid-binding proteins encoded by 5 distinct genes that mediate intermembrane movement of PtdIns through the aqueous medium. The PITP domain is described as a lipid binding cavity that consists of eight β-strands and seven α-helices; the β-strands form a large concave sheet flanked by two long α-helices. One well-described property of the single-domain PITP proteins is their ability to transport monomers of PtdIns or PtdCho between membrane compartments. This intermembrane transfer of PtdIns led to the naming of the protein as phosphatidylinositol transfer protein (PITP).



Thanks to PITPs and to membrane trafficking PtdIns is distributed to the cytosolic leaflet of multiple membrane-bound organelles where it can be processed by lipid kinases and phosphates

Phosphoinositide enzymes

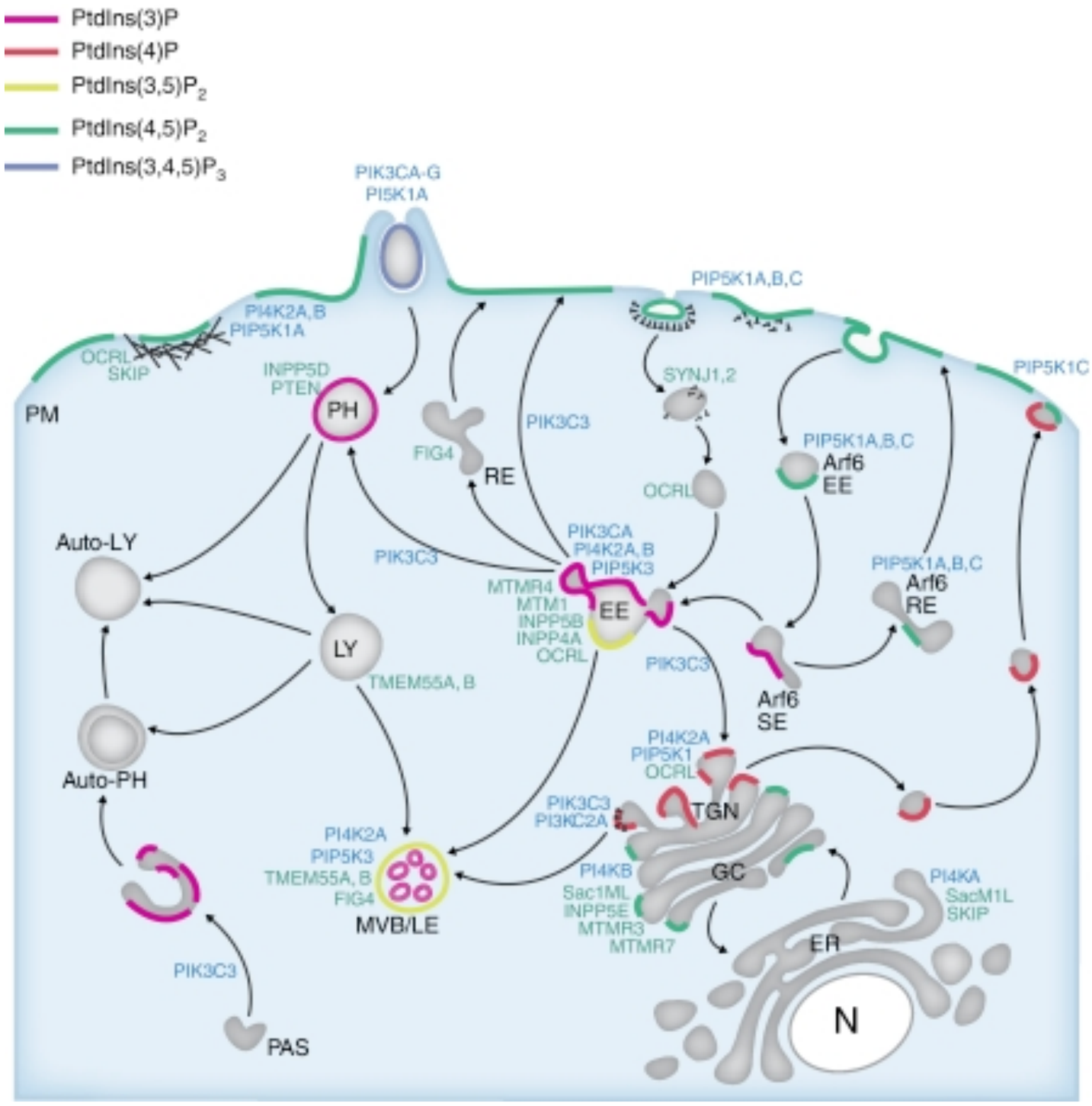


The phosphoinositides derive from reversible phosphorylation in three of the five hydroxyl groups of the inositol headgroup of the ‘parent’ PtdIns.

This process operates through the large repertoire of about 50 phosphoinositide kinases (PIKs) and phosphatases that are present in practically all cell compartments.

The combined activities of the various isoforms of these PIKs and phosphatases provide a dynamic equilibrium between the seven distinct, but interconvertible, PI species

Phosphoinositides

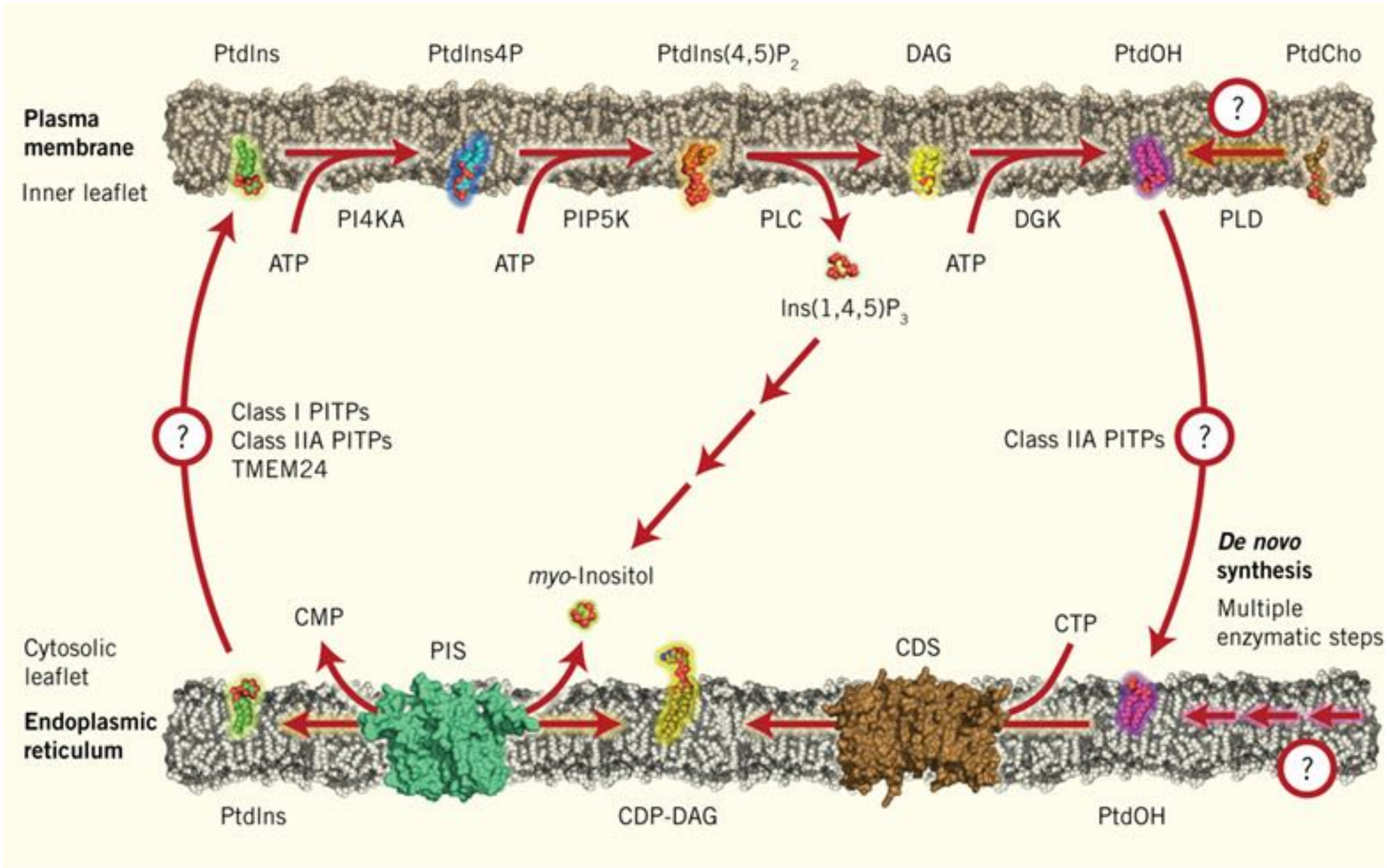


A distinctive feature of the organisation of phosphoinositide metabolism is its regionalisation, as opposed to the centralisation of most lipid biosynthetic pathways in the ER .

This derives from the fact that the e PIKs and phosphatases are different in each cellular compartment. This leads to the fact that at steady state, each of the seven phosphoinositide species maintain different concentrations across compartments.

Thus the dynamic interplay between PtdIns transport and processing leads to lipid compartmentalisation.

Phosphoinositides



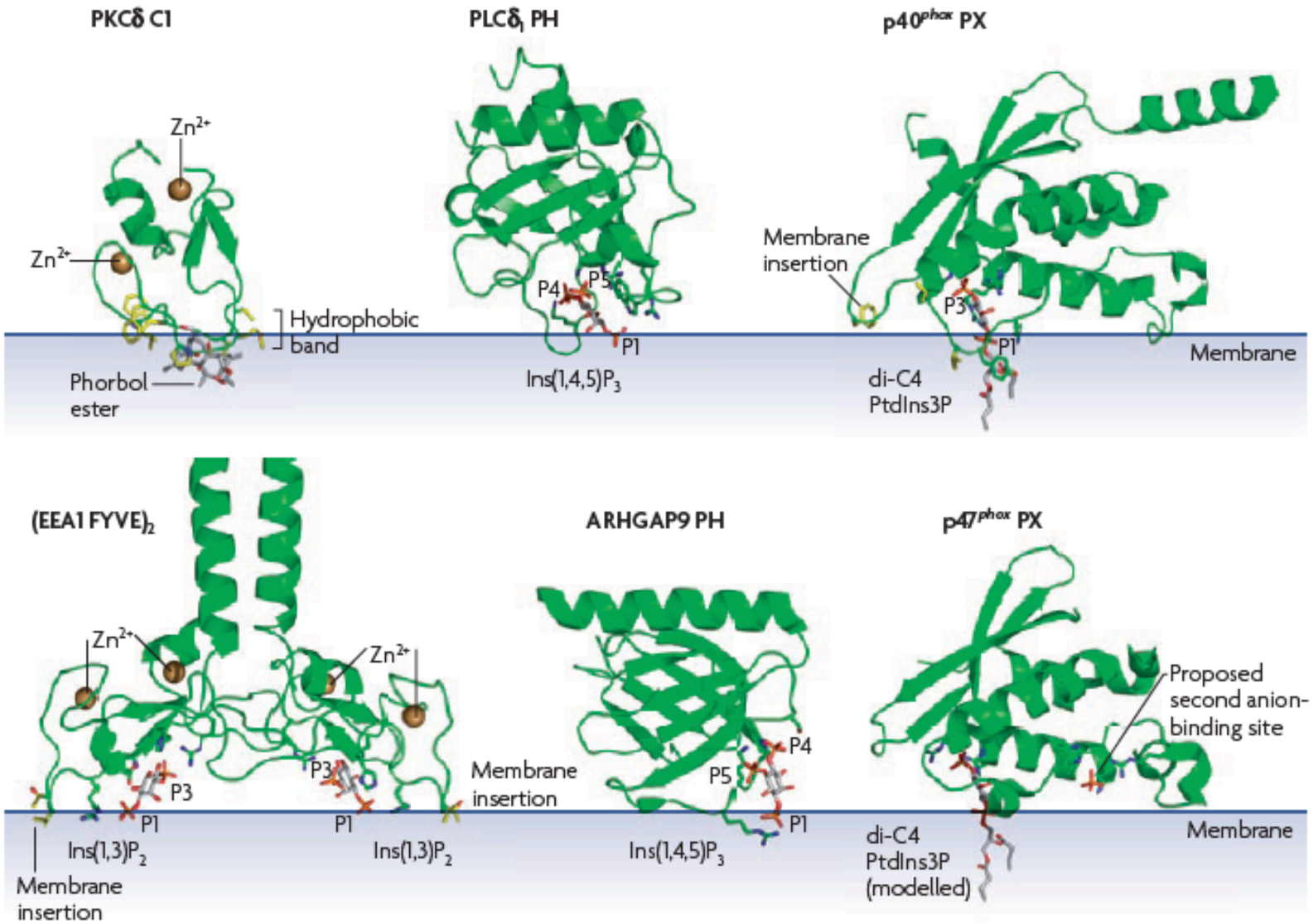
Phosphoinositides

A variety of domains that recognize specific phosphoinositides have been described and include pleckstrin homology (PH) domains, FYVE domains, and subsets of gelsolin homology domains, SH2 domains, and PTB domains. Some of these domains exist as large families, and specificity in binding is achieved by the ability of individual members of the family to recognize the phosphorylated moiety within distinct structural contexts.

Phosphoinositides

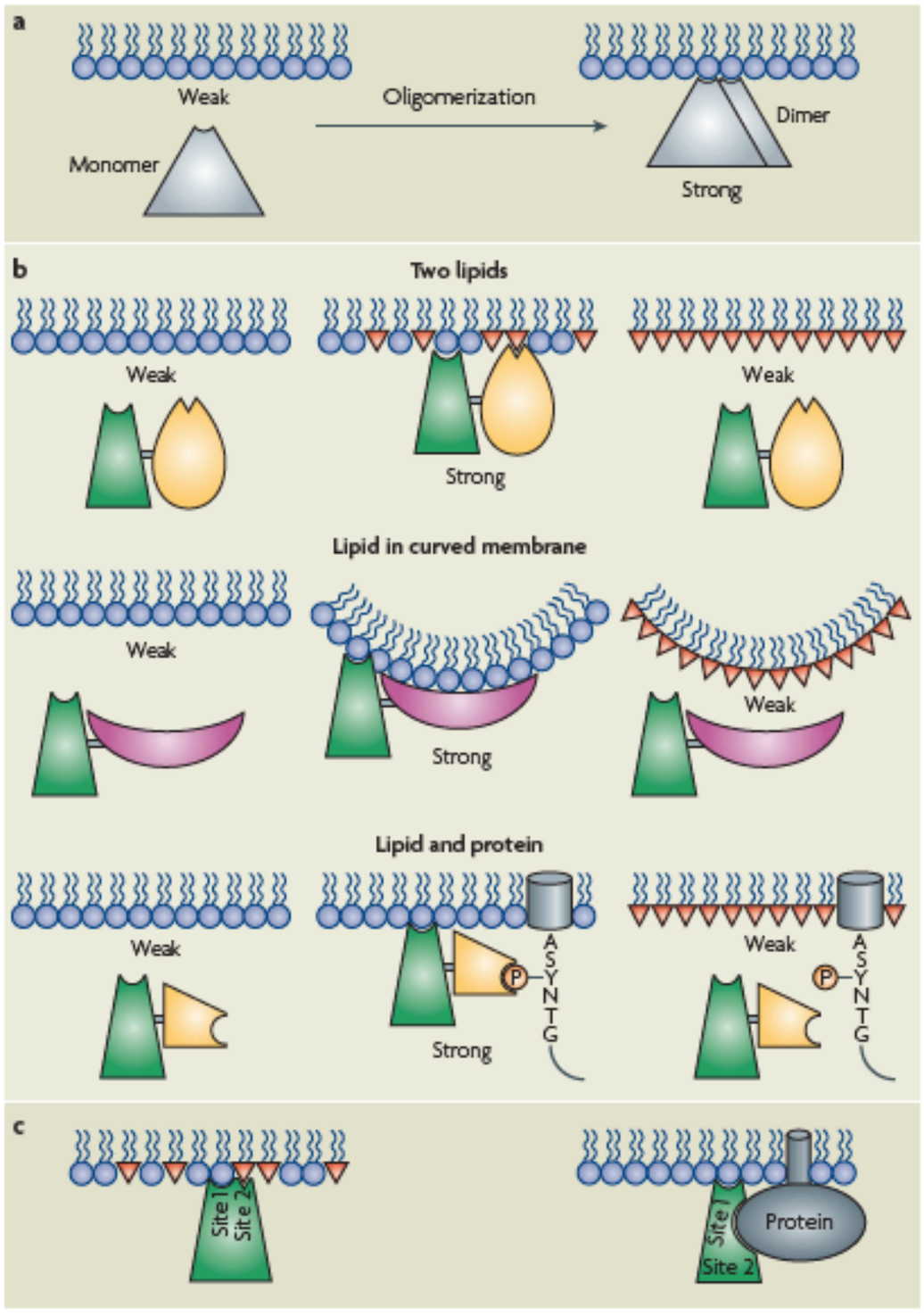
Domain	Typical size (amino acids)	Structure	Preferred target*	Membrane insertion?	Ca^{2+} required?	Dimerization required?
C1	~50	Zn^{2+} finger	DAG, phorbol esters	Yes	No	No
PKC C2‡	~130	β-sandwich	PtdSer (and others)	Yes	Yes	No
PH	~125	β-sandwich	Phosphoinositides, quite diverse, some highly specific	Some reported[20]	No	Some examples
FYVE	60–70	Zn^{2+} finger	PtdIns3P	Yes	No	Most cases
PX	~130	α+β structure	PtdIns3P (a few bind other phosphoinositides)	Yes	No	Most cases
PROPPIN	~500	β-propeller	PtdIns(3,5)P_2 (PtdIns3P also in some cases)	Unknown	No	No
Gla	~45	α-helical (requires Ca^{2+} to fold)	PtdSer	Yes	Yes	No
Annexin	~310	α-helical array	Acidic phospholipids	Unknown	Yes	No
Discoidin C2‡	~160	β-sandwich	PtdSer (specific)	Yes	No	No
ENTH	~150	α-helical solenoid	PtdIns(4,5)P_2 (some promiscuity)	Yes	No	No
ANTH	~280	α-helical solenoid	Phosphoinositides, relatively little specificity	No	No	Yes

Phosphoinositides



Phosphoinositide binding domains guide the membrane recruitment of the proteins where they are embedded. This together with the regionalised distribution of the different phosphoinositide species contributes to the organelle-specific recruitment of many cytosolic proteins that dynamically associate with membranes.

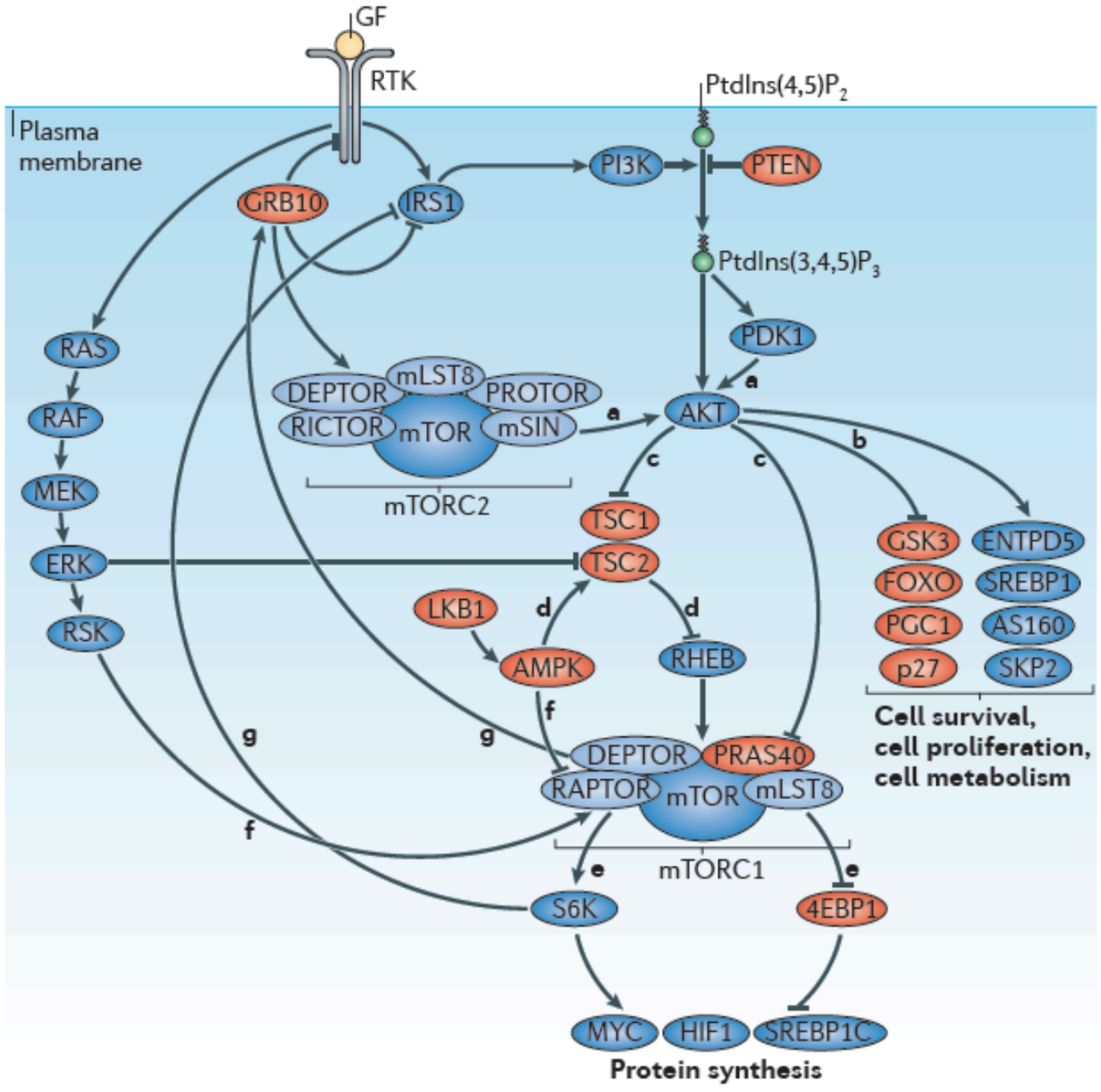
Coincident detection and organelle identity

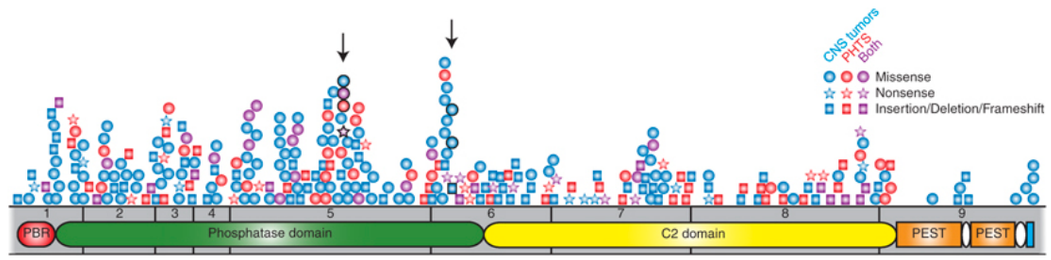


Phosphoinositide-binding domains commonly bind phosphoinositides with low affinities, favoring a highly plastic system whereby phosphoinositide-binding proteins continually sample the membrane environment, searching out and becoming enriched at sites of phosphoinositide presence. Weak binding is often enhanced through avidity effects (for example, as induced by oligomerization) and the ability to engage substrates in addition to the phosphoinositide lipid (**coincident detection**).

cells contain a diverse organelles, many of which are connected to each other by membrane traffic. These organelles perform specialized functions that require components to be sequestered from the cytoplasm. By defining the set of recruited proteins to a given organelle phosphoinositides (and their coincident detected factors) are said *encode* the **identity of that organelle.**

Phosphoinositide signalling

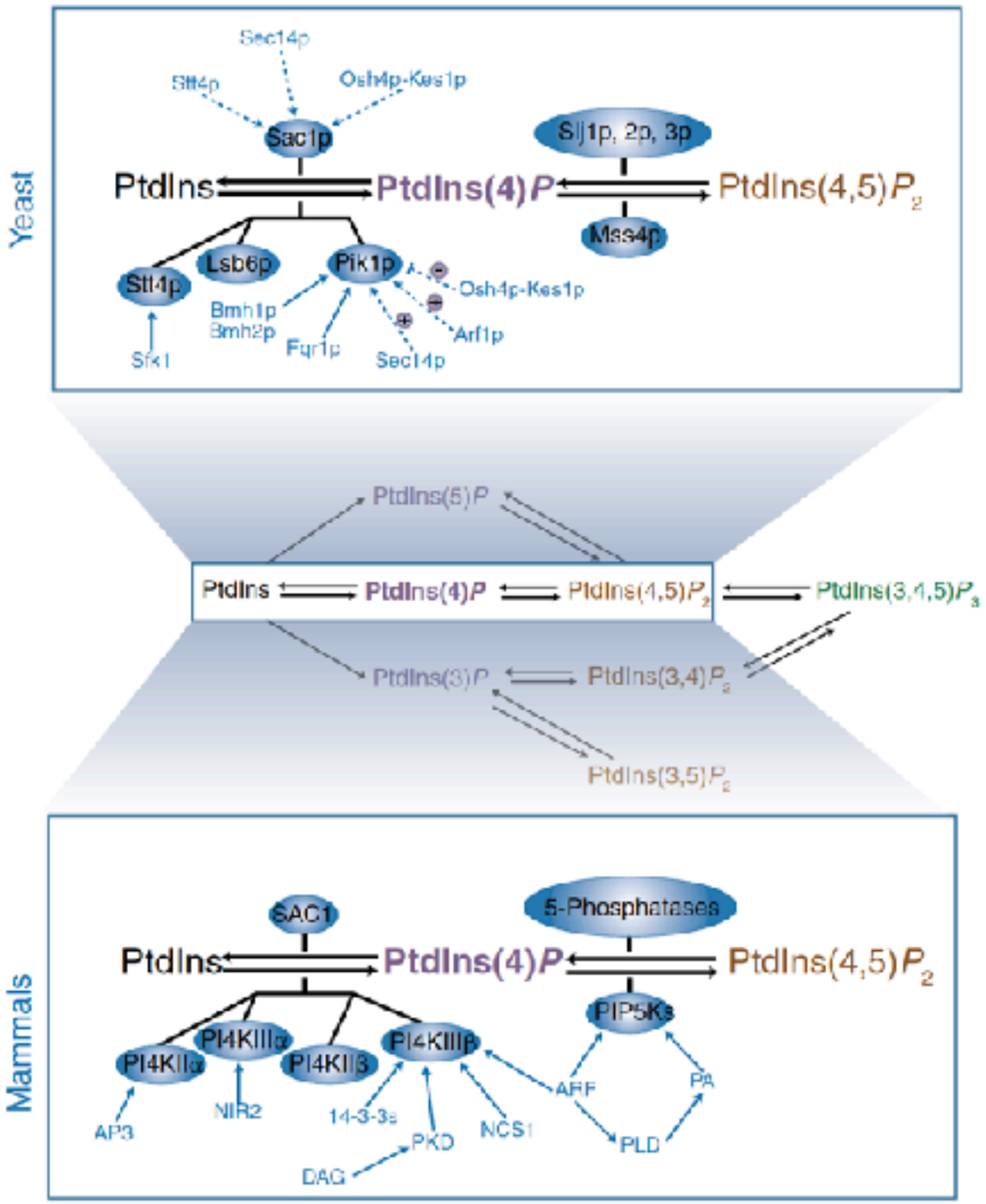




PTEN is one of the most commonly lost tumor suppressors in human cancer. During tumor development, mutations and deletions of PTEN occur that inactivate its enzymatic activity leading to increased cell proliferation and reduced cell death. Frequent genetic inactivation of PTEN occurs in glioblastoma, endometrial cancer, and prostate cancer; and reduced expression is found in many other tumor types such as lung and breast cancer.

"Up to 70 percent of men with prostate cancer have lost one copy of the PTEN gene at the time of diagnosis".

PtdIns(4)*P* - a lipid metabolic crossroad

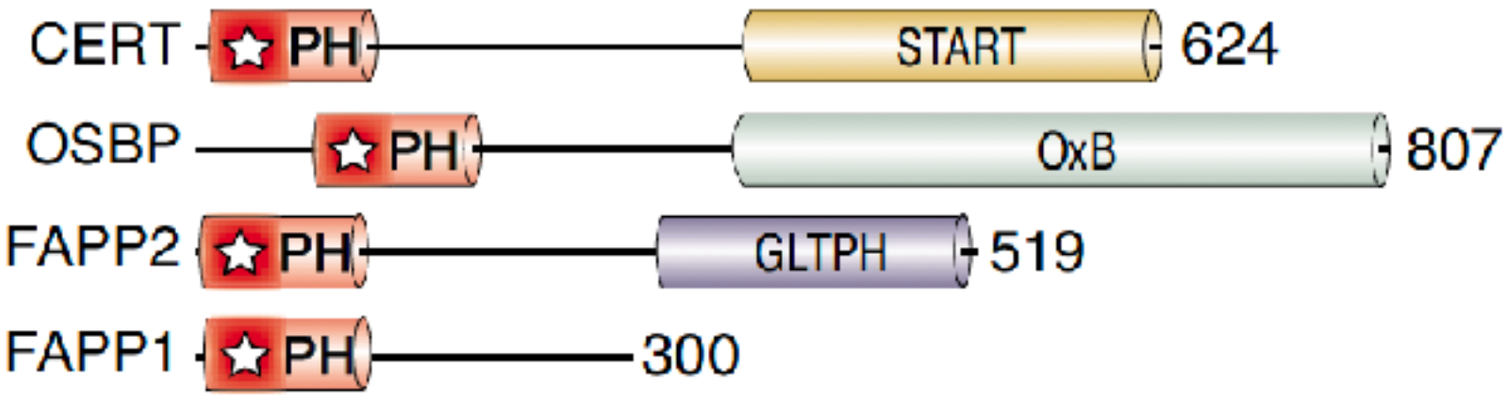


PtdIns(4)*P* is the most abundant of the mono-phosphorylated phosphoinositides. It is produced from PtdIns by the action of **PI4Ks** some of which are active at the Golgi complex and turned over by the ER localized **Sac1**.

As a consequence of these enzyme distributions PtdIns(4)*P* is more abundant in the Golgi (and specifically in the trans Golgi) than in the ER.

Enzyme	Localisation	Molecular mass (kDa)	Gene
PI 4-kinases			
Mammalian			
PI4KIIα	Golgi, TGN, PM, endosomes, synapse	55-56	*PI4K2A, 10q24*
PI4KIIβ	Golgi, TGN, PM, endosomes, ER	55-56	*PI4K2B, 4p15.2*
PI4KIIIα	PM, ER, nucleus	210	*PI4K2A, 22q11.21*
PI4KIIIβ	Golgi, TGN, nucleus, endosomes, exocytic vesicles	110	*PI4KB, 1q21*
Yeast			
Lsb6p	PM, vacuole	70	*LSB6, X*
Stt4p	PM	216	*LSB6, X*
Pik1p	Golgi, nucleus	125	*STT4, XII*
PI 4-phosphatases			
Mammalian			
Sac1	ER, Golgi	64	*SACM1L, 3p21.3*
Synaptojanin 1	Clathrin-coated vesicles (nerve terminals)	145-170	*SYNJ1, 21q22.2*
Synaptojanin 2	Clathrin-coated vesicles (ubiquitous)	140	*SYNJ12, 6q25.3*
Yeast			
Sac1p	ER, Golgi	67	*SAC1, XI*

PtdIns(4)*P* - a lipid metabolic crossroad



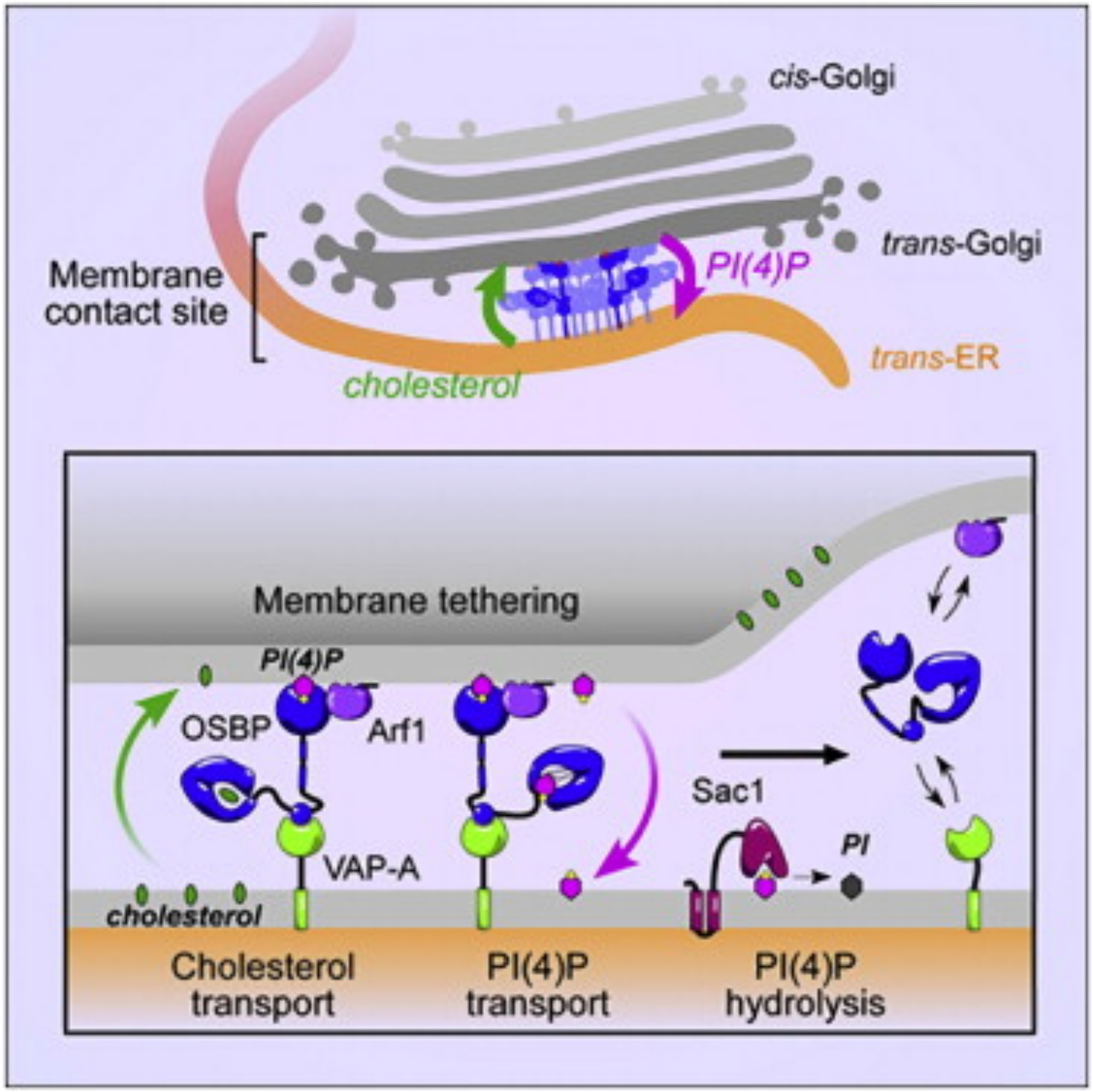
At the *trans* Golgi PtdIns(4)*P* recruits a family of lipid transfer proteins involved in sphingolipid metabolism (i.e., CERT1 and FAPP2) as well as the cholesterol transfer protein OSBP1.

CERT, FAPP2, and OSBP1 together deliver ceramide, GlcCer and cholesterol to the *trans* Golgi where ceramide and GlcCer are processed to produce sphingomyelin and glycosphingolipids.

Sphingomyelin, glycosphingolipids and cholesterol are major constituents of the extracellular leaflet of the plasma membrane.

Thus by recruiting its effectors to the *trans*-Golgi, PtdIns(4)*P* determines the lipid composition of the post Golgi compartments.

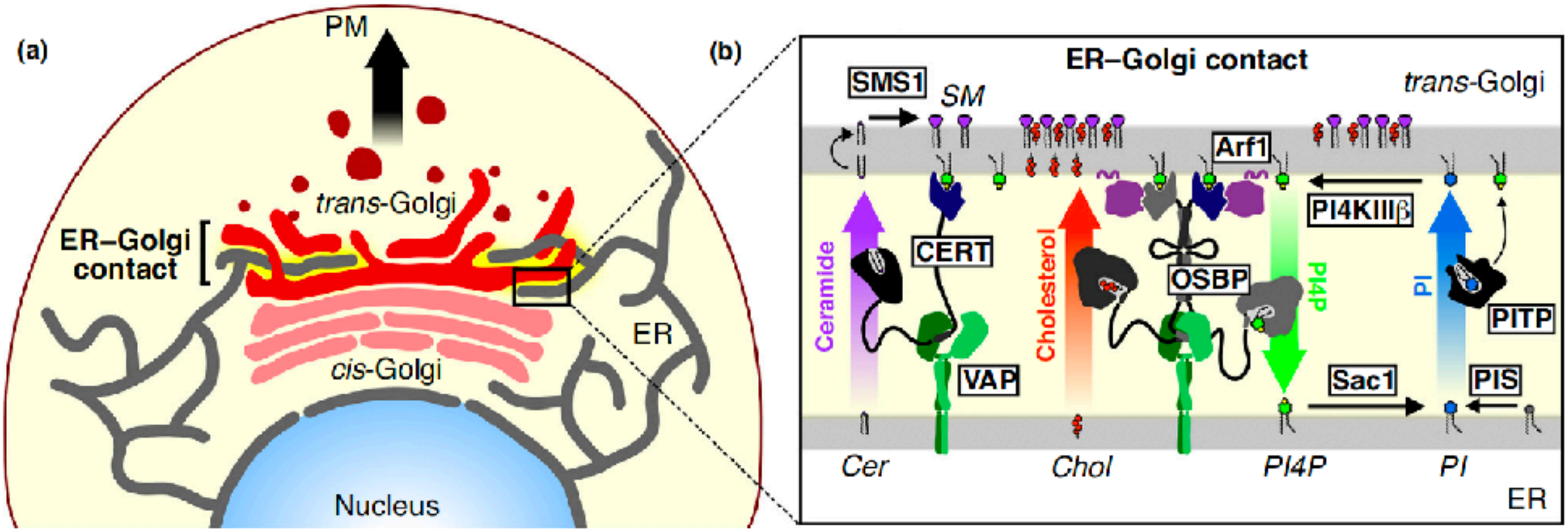
PtdIns(4)*P* - a lipid metabolic crossroad



Interestingly OSBP1 has an additional activity. On top of transferring cholesterol from the ER to the *trans*-Golgi it transport PtdIns(4)*P* from the *trans*-Golgi back to the ER where it gets dephosphorylated by the Sac1 phosphatase.

Thanks to this dual activity OSBP1 is a self limiting machine that consumes its recruiting factor [i.e., PtdIns(4)*P*] while pumping cholesterol to the *trans*-Golgi

PtdIns(4)*P* - a lipid metabolic crossroad



Take home messages

- Ceramide (the common sphingolipid precursor) can be produced *de novo* through a series of 4 reactions or by a salvage pathway in the ER.

- Sphingomyelin and glycosphingolipids are synthesised at the Golgi through pathways that require the action of lipid transfer proteins.

- Phosphoinositides are phosphorylated derivatives of PtdIns that are distributed unevenly across subcellular membranes.

- Phosphoinositides recruit proteins to specific membranes to trigger signalling events, to determineorganeelle identity and to modulate lipid metabolic fluxes.