



Lipid droplets formation and their effects on phosphatidylinositol level in yeasts

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Abstract

Lipid droplets (LDs) are conserved specialized organelles that are surrounded by a single phospholipid layer, enclosing neutral lipids in form of triacylglycerol (TAG) and/or sterol ester. They are formed at the endoplasmic reticulum (ER), and stay connected to it in the yeast cell. These LDs mainly play major roles in lipid storage and homeostasis. Moreover, they have several functions in the yeast cell including; protein degradation, protein glycosylation, response to ER stress, in addition to providing substrate for energy metabolism and for membrane synthesis. In general, TAG and phosphatidylinositol (PI) synthesis share the same precursor; phosphatidic acid (PA), which controls their level. By the actions of lipases, phosphatases and/or kinases; one can act as a precursor for another, thus explaining the reason of their inverse correlation. TAG lipolysis is catalyzed by TAG lipases producing fatty acids and diacylglycerol (DAG). The DAG produced from lipolysis is phosphorylated by diacylglycerol kinase (Dgk1) to form PA. On the other hand, PA is a cell signaling molecule and once increased, it upregulates cytidine diphosphate diacylglycerol (CDP-DAG) pathway for the PI synthesis. Major TAG lipases are mainly localized in LD, whereas minor amounts of them are localized in ER. Once DAG is produced inside the LD, it is transferred to the ER by inheritance cortical ER protein, Ice2, for further reactions. The TAG and PI levels are also controlled by transcriptional regulation in response to inositol and zinc. The aim of this study was to clarify how LDs regulate lipid homeostasis in the yeast cell, and particularly focuses on the balance between TAG and PI.

Keywords: Endoplasmic reticulum, Lipid droplets, Phosphatidylinositol, *Saccharomyces cerevisiae*, Triacylglycerol

1. Introduction

Lipids are involved in several biological processes in the cells including: formation of the cell membrane; which insulates the cell interior protecting it from the surrounding environment, storage and production of

energy within the cell, cell signaling, facilitating cell division by providing membrane flexibility and mediating the cell fusion and apoptosis ([Kimura et al., 2016](#)). According to [Fahy et al., \(2011\)](#), lipids are

classified into 8 classes which are Glycerolipids, glycerophospholipids, sphingolipids, sterol and sterol derivatives, polyketides, glycolipid, prenol lipids and fatty acids (FA).

Previous studies of [Ploegh, \(2007\)](#); [Klug and Daum, \(2014\)](#) highlighted that fatty acids are the building blocks of membrane lipids such as phospholipid, and of storage lipids such as TAG and steryl ester. An early work of [Gaigg *et al.*, \(2001\)](#) documented that yeast cells get FA from three different sources mainly; *de novo* synthesis, hydrolysis of complex lipid and proteins de-lipidation, and finally from external sources through FA uptake.

A recent study of [Wang *et al.*, \(2018\)](#) revealed that deregulation of lipids within a cell causes toxicity, and ends up with triggering the cell death through different ways including prolonged activation of signaling pathways such as the unfolded protein response (UPR) ([Olzmann and Carvalho, 2019](#)). Lipid regulation, assembly, storage and mobilization are controlled by intracellular organelles called lipid droplets (LDs), as reported by [Pol *et al.*, \(2014\)](#).

LDs are conserved dynamic and functional organelles that regulate lipid homeostasis through the storage and hydrolysis of neutral lipids. In yeast cells, LDs are formed from ER and stay connected to it. [Listenberger *et al.*, \(2003\)](#) revealed that the storage of neutral lipids in LDs is crucial for the cellular defense against lipotoxicity. LDs store neutral lipids in the form of TAG and sterol ester, and their formation is coupled with the synthesis of one or both of them. LDs are not formed in cells with no neutral lipids ([Olzmann and Carvalho, 2019](#)). In addition to the LDs roles in lipid homeostasis, they also participate in protein glycosylation ([Krahmer *et al.*, 2013](#)), protein degradation ([Hartman *et al.*, 2010](#)), response to ER stress ([Fei *et al.*, 2009](#)), and promote infection by viral, protozoan and bacterial pathogens ([Wilfling *et al.*, 2014](#)). Moreover, LDs provide substrates for energy metabolism, membrane synthesis and in production of essential lipid-derived molecules including; hormones, bile salts and lipoproteins ([Pol *et al.*, 2014](#)).

According to the recent study of [Yeagle, \(2016\)](#), phosphatidylinositol (PI) is a major phospholipid in addition to phosphatidyl choline (PC), phosphatidyl ethanolamine (PE) and phosphatidyl serine (PS); and composes up to 10 % of the total phospholipid in the eukaryotic cells.

Both TAG and PI biosynthesis takes places in ER; however, the last step of synthesis of some amounts of TAG is completed in LDs. TAG is built up of three fatty acids and a glycerol, while PI is built up of two fatty acids, a glycerol and a phosphate group with an inositol ring (Fig. 1). According to [Adeyo *et al.*, \(2011\)](#); [Henry, \(2012\)](#), both of TAG and PI are synthesized from the same precursor; PA and one can be converted to the other by the actions of lipases, phosphatases and kinases. Recent reports of [Van der Veen *et al.*, \(2017\)](#); [Wang *et al.*, \(2018\)](#); [Wang *et al.*, \(2019\)](#) proved that there is an inverse correlation between TAG and PI, where an increase of TAG level is accompanied by a decrease of the PI level and vice versa.

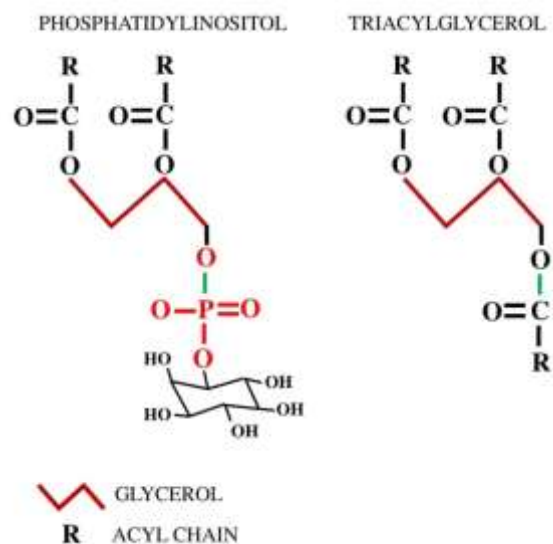


Fig. 1. Structures of PI and TAG. PI consists of two fatty acids, a glycerol and a phosphate with an inositol ring. However, TAG is built by three fatty acids and a glycerol. Through the actions of phospholipase C or TAG lipases (on the green bond), one can be a precursor of another.

In this review we focused on TAG synthesis, LD biogenesis, TAG and PI inverse correlation, and discussed the mechanism of regulation of PI by TAG synthesis, storage and degradation, to clarify how phospholipids and storage lipids are regulated.

2. TAG synthesis and LDs biogenesis

2.1. TAG synthesis

Phosphatidic acid (PA) is the initial precursor of the synthesis of all phospholipids and TAG (Fig. 2). PA is synthesized via two step acylation reactions from glycerol-3-phosphate (G3P). [Zheng and Zou, \(2001\)](#) demonstrated that Sct1 and Gpt2 acyltransferases, catalyze the first acylation reaction, giving sn1-acylglycerol-3-phosphate (also termed lyso-PA) as a product. Later, [De Smet *et al.*, \(2012\)](#) reported that Sct1 and Gpt2 can also acylate dihydroxyacetone phosphate (DHAP) to give 1-acyl-DHAP, which is reduced later by Ayr1 reductase to give lyso-PA. Previous works conducted by [Jain *et al.*, \(2007\)](#); [Riekhof *et al.*, \(2007\)](#) revealed that the second reaction is the acylation of lyso-PA by Slc1 and Ale1 acyltransferases to form sn1, 2-diacylglycerol-3-phosphate also called PA. [Carman and Han, \(2018\)](#) revealed that TAG synthesis starts from de-phosphorylation of PA by phosphatidate phosphohydrolase (Pah1) to make diacylglycerol (DAG) This DAG is then converted to TAG by other acyltransferases.

There are two acyltransferases that synthesize TAG from DAG; a phospholipid dependent acyltransferase (Lro1); and acyl-Coenzyme A (CoA) dependent acyltransferase (Dga1). In contrast to Lro1 that is strictly localized in the ER, Dga1 has two trans-membrane segment hairpin like structure, which allows it to be localized in the ER and LDs. All TAG that are synthesized at LDs are mediated by Dga1 ([Markgraf *et al.*, \(2014\)](#)).

2.2. LD biogenesis

Although several models for LD biogenesis have been suggested by [Guo *et al.*, \(2009\)](#), the lensing

model (Fig. 3) is widely accepted and is experimentally supported by [Brasaemle and Wolins, \(2012\)](#). In this model, the neutral lipids are synthesized, deposited and coalesce between the ER leaflets. After the deposited lipids reach a critical size, LD bud is formed toward ER cytosolic side with neutral lipid enclosed in it. In yeasts, LD stays attached to ER membrane ([Jacquier *et al.*, \(2011\)](#)).

Initially neutral lipids are synthesized at low concentration in the ER and stay dispersed between the two ER leaflets. [Thiam and Forêt, \(2016\)](#) added that as the concentration of neutral lipids increases up to the range between 5-10 mol%, they start to coalesce to form an oil lens through a de-mixing process. Expansion of the neutral lipid lens causes membrane tension and results in the formation of LD bud from the ER membrane. Recently, [Chen and Goodman, \(2017\)](#) reported that this budding process is not well understood, but several proteins that are involved in the process have been elucidated including; seipin complex, Pex30, Fit2 related proteins and Pet10.

[Walther *et al.*, \(2017\)](#) demonstrated that seipin protein complex plays a role in promoting the initiation of nascent LD formation, by marking the sites where TAG accumulates. It also facilitates the growth and the expansion of LD by enabling lipid transfer from the ER to nascent LD. This process results in the maturation of LDs. Moreover, seipin complex controls the morphology, the number and the size of LDs.

Ldo proteins are recently identified by [Teixeira *et al.*, \(2018\)](#) as subunits of the seipin complex; they are expressed as two proteins, Ldo45 and Ldo16 with a unique splicing. Ldo genes deletion affects some of the LD proteins localization. [Teixeira *et al.*, \(2018\)](#) added that overexpression of Ldo45 increases the TAG level up to 3 folds more than the wild-type, by dampening the lipolysis and increasing the Dga1 mediated synthesis. The effects of Ldo45 can be regarded in TAG level regulation, protein localization and LD morphology, whereas Ldo16 plays a role in lipophagy. [Teixeira *et al.*, \(2018\)](#) reported that during the stationary phase there are changes in cell lipid

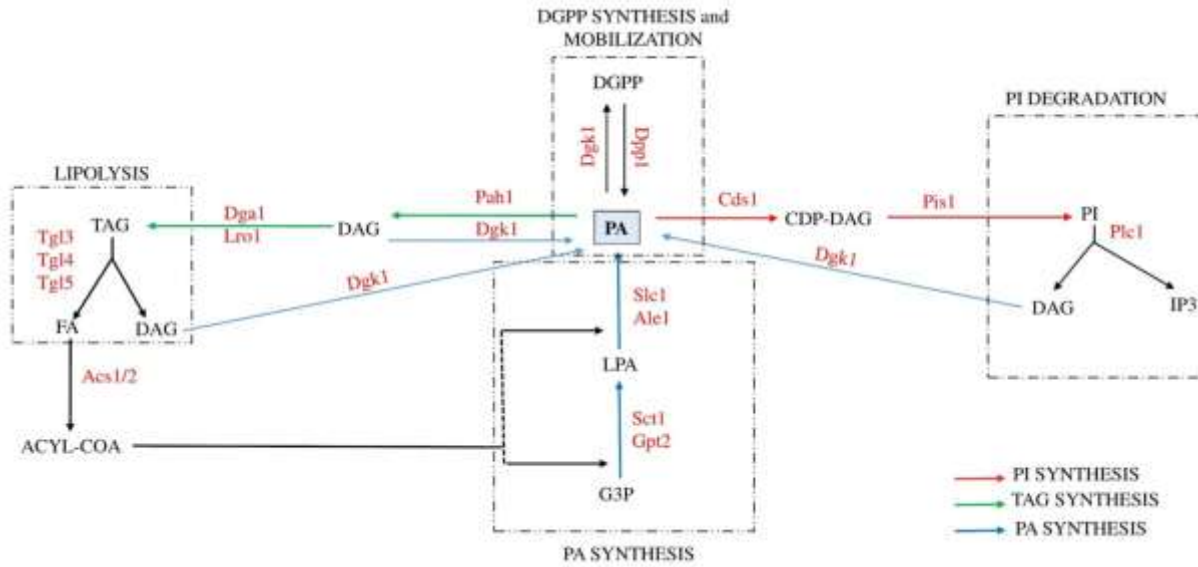


Fig. 2. Phosphatidic acid (PA) synthesis and degradation. PA is utilized as a substrate for TAG and PI synthesis, and their degradation gives DAG as a product, which is later phosphorylated by Dgk1 to synthesize PA

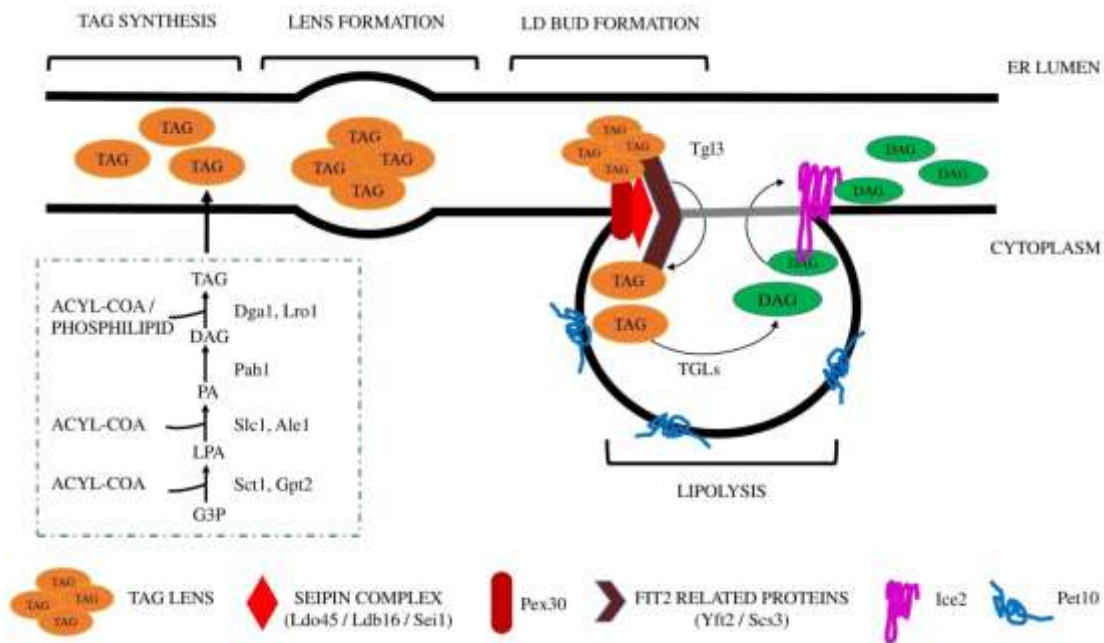


Fig. 3. Schematic representation of LD biogenesis and TAG lipolysis. As the concentration of TAG increases in the ER membrane, the synthesized TAGs coalesce and form a lens. Seipin complex and Pex30 mark the sites, where the TAG lens accumulates for a nascent LD formation. FIT2 related proteins transfer TAGs from ER to the LD. TAG is converted to DAG and FA through the actions of TAG lipases localized in LD. The DAGs produced during lipolysis are transferred back to the ER by Ice2. Pet10 is recruited to the nascent LD from the cytosol for membrane tension balance.

composition and nutrient level. One of the changes in lipid composition is the increase in storage lipid, which later becomes an energy source through a specific form of micro autophagy called lipophagy. For energy production, the LDs that are normally localized in the proximal region of the vacuole during the stationary phase translocate eventually to the vacuole lumen for degradation. In *ldo16Δ* cells, LDs are localized as the same to both the wild-type and *ldo45Δ*, but fail to translocate to vacuole for lipophagy. This is also supported with an accumulation of TAG in the LDO16 deleted cells, suggesting a role of Ldo16 in lipophagy.

Recently, [Wang *et al.*, \(2018\)](#) revealed that an ER protein Pex30 is required for LD budding in seipin deleted cells. This Pex30 co-localizes with nascent LDs and LD-proteins including seipin ([Wang *et al.*, 2018](#); [Nettebrock and Bohnert, 2020](#)). The *pex30Δ* mutant shows a mild delay in LD budding, and a dramatic increase in DAG, PE and PI levels have been observed in *pex30Δ*, *sei1Δ* and *ldb16Δ*. This suggests a role of Pex30 in LD budding, in addition to the inverse correlation between TAG and PI.

[Kadereit *et al.*, \(2008\)](#) highlighted that among the other proteins that are involved in LD budding, there are human homologies with the fat storage inducing trans-membrane (FIT) proteins. In general, the human cells have two FIT proteins namely, FIT1 and FIT2. Whereas, the yeast cells have no FIT1 homologue, but have two FIT2 homologous proteins mainly, Yft2 and Scs3. Later, [Gross *et al.*, \(2011\)](#); [Choudhary *et al.*, \(2018\)](#) added that the FIT2 related proteins are ER resident proteins that are involved in partitioning the neutral lipids from ER for LDs formation. This point is supported by the fact that a purified human FIT2 binds DAG and TAG *in vitro*, accompanied by an increase of DAG and TAG in the ER of the *fit2* deficient cells. [Gross *et al.*, \(2011\)](#); [Choudhary *et al.*, \(2015\)](#) works demonstrated that an overexpression of FIT2 related proteins results into larger LDs, but it doesn't affect the level of TAG or inhibits lipolysis. However, its

deletion results into smaller LDs in addition to the accumulation of neutral lipids lenses in the ER, thus suggesting its role in TAG partitioning. According to [Gross *et al.*, \(2011\)](#), the FIT2 related proteins also ensure the directionality of LD budding, and make sure that a nascent LD grows towards the cytosolic side rather than the luminal side of the ER. Previous works of [Choudhary *et al.*, \(2015\)](#); [Wang *et al.*, \(2016\)](#) suggested that on deletion of the FIT2 related proteins, LDs become embedded in the ER. Moreover, they showed an abnormal ER morphology with several membrane clumps, thus suggesting their roles in LD bud directionality.

Recently, [Gao *et al.*, \(2017\)](#); [Olzmann and Carvalho, \(2019\)](#) reported that Pet10 is a member of the perilipin protein family, which is known to function in protecting LDs from lipase activity. In addition, it collaborates with the FIT2 related proteins and seipin complex for LD budding formation. This Pet10 facilitates LD budding by changing the balance tension between the two ER membrane monolayers.

3. Relationship between LD biogenesis and PI level

The PI level changes by mutations in the enzymes involved in TAG synthesis, hydrolysis and/or LD biogenesis. Both of the TAG and PI are synthesized from the same precursor, PA. Therefore, there is a competition between them and one can be changed to the other. Several reports of [Henry *et al.*, \(2012\)](#); [Markgraf *et al.*, \(2014\)](#) revealed their inverse proportionality and regulation.

3.1. PA conversion

Phosphatidic acid (PA) is a common precursor for both of TAG, PI and other phospholipids. It can be converted into DAG by Pah1 or into CDP-DAG by CDP-Diacylglycerol Synthase (Cds1), through Kennedy or CDP-DAG pathways, respectively. Some phospholipids such as PC and PE can be synthesized through both pathways, while others including PS and

PI use only the CDP-DAG pathway, as highlighted by [Henry *et al.*, \(2012\)](#); [Graef, \(2018\)](#). Conversion of PA is the most important regulatory step for lipid biosynthesis. Competition between Cds1 and Pah1 determines the levels of TAG and PI. The CDP-DAG pathway, which is essential for PI synthesis, starts with the condensation of PA and cytidine triphosphate (CTP); eliminating pyrophosphate to give CDP-DAG, this reaction is catalyzed by Cds1. Finally, PI synthesis is completed through a phosphatidylinositol synthase (Pis1), which catalyzes the binding of inositol-3-phosphate (synthesized by Ino1) to the CDP-DAG ([Loewen, 2004](#)). A study conducted by [Qi *et al.*, \(2016\)](#) reported that deletion of CDS1 in a mammalian cell (where the gene is not essential) increases the PA level dramatically in ER, and consequently give rise to a super-sized LD. On the other hand, the Kennedy pathway starts with PA dephosphorylation by Pah1, which converts PA into DAG. While, the Lro1 or Dgal acyl-transferases convert DAG to TAG. In *pah1Δ* cell, the level of TAG is significantly decreased and ER/nuclear membrane is abnormally expanded, thus suggesting the increase in phospholipids ([Barbosa *et al.*, 2015](#)).

A previous work of [Han *et al.*, \(2008\)](#) highlighted that Dgk1 is an enzyme with opposite activity to Pah1, it converts DAG into PA. Deletion of Dgk1 in *pah1* mutant cell rescues the abnormal ER/nuclear membrane expansion, and result in a reduction of LD number observed in a *pah1Δ* strain. Meanwhile, an overexpression of Dgk1 results in expansion of the nuclear/ER membrane due to the increase in phospholipids synthesis, whereas its deletion causes a decrease in the PA level. [Han *et al.*, \(2008\)](#) reported that the function of Dgk1 in the nuclear membrane counteracts that of Pah1 through controlling the cellular levels of PA. In addition, an increase in the PA levels up-regulates the transcription of PI biosynthetic genes.

3.2. Actions of the TAG lipases

Previous works of [Rajakumari *et al.*, \(2010\)](#); [Gaspar *et al.*, \(2011\)](#) highlighted that TAG lipases are

encoded by TGL genes, and they catalyze the conversion of TAG to DAG. In wild-type cells, the TAG lipases are recruited from the ER to LDs. However, in mutant cells that are devoid of LDs, TAG lipases are accumulated in the ER and lose their lipolytic activity. Lipolysis of TAG by TAG lipases gives rise to DAG and fatty acids, which serves as precursors for the synthesis of the membrane phospholipids including PI. Yeast cell has 5 TAG lipases namely, Tgl1, Tgl2, Tgl3, Tgl4 and Tgl5. They are localized in the LD, except for Tgl2 which is localized in the mitochondria. [Ham *et al.*, \(2010\)](#) revealed that in cells devoid of LDs, the TAG lipases are localized in the ER, and are partially retained in the ER in cells where TAG is not synthesized. Previous reports of [Schmidt *et al.*, \(2013\)](#) showed that minor amounts of Tgl3 are detected in the ER. While Tgl1 is specific to sterol ester, and its deletion does not affect the mobilization of TAG ([Köffel *et al.*, 2005](#); [Klein *et al.*, 2016](#)). Tgl3, Tgl4 and Tgl5 are the major TAG lipases in yeast cells. [Athenstaedt and Daum, \(2005\)](#) demonstrated that *tgl3Δ*; *tgl4Δ* double mutant cells show an increased number and size of the LDs. The same authors added that Tgl3 preferentially hydrolyzes TAG species containing C14, C16, C20, and C26 saturated acyl chains, whereas cells defective in Tgl5 show an increased level of TAG containing C26 acyl chain. The triple mutant cells mainly; *tgl3Δ*, *tgl4Δ*, *tgl5Δ* are able to synthesize TAG, but not able to mobilize it. In these mutant cells, the PI level is decreased as reported by [Gaspar *et al.*, \(2011\)](#), thus suggesting a direct link between TAG breakdown and the increase in level of the PI. Later, [Markgraf *et al.*, \(2014\)](#) highlighted that both products of TAG lipolysis namely; DAG and FA, are needed for the synthesis of PI.

3.3. Proteins involved in LD formation

LD buds emerge from the cytosolic side of the ER, catalyzed by Sei1, Pet10, Pex30 and FIT2-related proteins ([Wang *et al.*, 2018](#); [Nettebrock and Bohnert, 2020](#)). Deletion of any of the genes encoding proteins involved in LD bud formation, affect the cellular TAG level. Several works of [Han *et al.*, \(2015\)](#); [Chen and](#)

[Goodman, \(2017\)](#) demonstrated that deletion of seipin complex reduced 50% of the TAG, deletion of FIT2 related proteins caused 15% reduction, whereas that of Pet10 resulted in 35% drop in the TAG level. [Teixeira et al., \(2018\)](#) study reported that overexpression of Ldo45 causes 3-4 folds increase in TAG compared to the wild-type; however, an opposite phenotype is detected in *pet10Δ* mutant ([Gao et al., 2017](#)). Recent work of [Velázquez et al., \(2016\)](#) revealed that cells with no LD have relatively increased level of PI and decreased level of PA.

3.4. FIT2 related proteins, TAG lipases, Ice2 and Dgk1 interaction

[Markgraf et al., \(2014\)](#) reported that transfer of yeast cells from the stationary phase with high TAG level to a fresh medium, results in a decrease in the TAG level and in an increase in PA and PI. Meanwhile, the TAG lipases, Dgk1, Ice2 and FIT2 related protein interact with this increase in the PI level.

During LD formation, FIT2 related proteins transfer TAG and DAG from ER to LDs for storage. The TAG imported to or synthesized at the LDs is broken down by TAG lipases through a lipolysis reaction, producing FA and DAG inside the LDs. This DAG produced in the LDs are transferred back to ER by the Ice2, where they are further phosphorylated by Dgk1p to form PA. Such increase in the level of PA activates the CDP-DAG pathway for PI synthesis ([Markgraf et al., 2014](#)).

Ice2 is a multi-spanning membrane protein localized in the ER during exponential growth of the cells, and in the proximal LDs at the stationary phase ([Markgraf et al., 2014](#)). This Ice2 acts as a DAG transferring protein from LDs to the ER, and delivers DAG to Dgk1. Such DAG transferred to the ER cannot be phosphorylated by Dgk1 in the absence of Ice2. The *ice2Δ* mutant cells have a delay in lipolysis which is almost at the same level to *tgl3Δ*. During growth resumption, the TAG level in *ice2Δ* is 3 folds higher compared to the wild-type. Moreover, [Markgraf et al., \(2014\)](#) added that on comparing the stationary and

growth resumption phase in the *ice2Δ* cells, the PS and PI level in the cell remained the same while the DAG level remained high, confirming the DAG channeling function of the Ice2. These *ice2Δ* mutant cells undergo a futile cycle in LDs, where TAG is continuously degraded by the TAG lipases to DAG, and then resynthesized by Dga1.

4. Transcription, regulation of PI and TAG biosynthesis

Several genes that are involved in phospholipid synthesis in yeasts are sensitive to the inositol level, and are regulated by the inositol transcriptional regulation (Fig. 4). They share a common cis-acting element 5'CATGTGAAT 3' in their promoters, which is known as inositol sensitive upstream activating element, (UAS_{INO}). The transcription activator, Ino2-Ino4 heterodimer, binds on the UAS_{INO} element, for their transcriptional activation ([Bachhawat et al., 1995](#)). The transcription of genes under the UAS_{INO} element such as *CDS1* and *INO1* is repressed by Opi1; a transcription repressor that binds with Ino2 transcription activator and repress the transcriptional activation.

According to [Loewen et al., \(2004\)](#), the PA level is a key regulator of the transcriptional activation and repression of the UAS_{INO} containing genes. [Henry, \(2012\)](#) reported that under growth conditions such as; inositol starvation, zinc supplementation or exponential phase in which the PA is relatively high, Opi1 binds on the ER/nuclear membrane where it stays inactive outside the nucleus. Opi1 then binds with an integral ER membrane protein, Scs2, through the FFAT motif, and with PA via its PA binding domain. [Dietz et al., \(2003\)](#); [Henry, \(2012\)](#) added that inactivation of the transcription repressor results in the active transcription of the UAS_{INO} containing genes. Once the PA level is decreased, Opi1 dissociates from ER/nuclear membrane and then translocate to the nucleus, where it represses the transcription of UAS_{INO} containing genes by binding with Ino2. The PI level is also regulated by zinc availability.

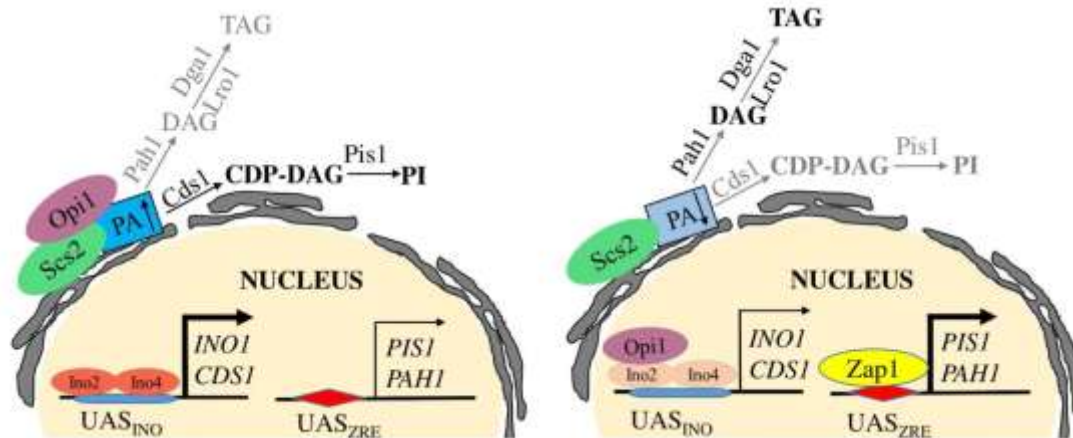


Fig. 4. Transcriptional regulation of the genes involved in PI and TAG biosynthesis. The transcriptional activation of UAS_{INO} containing genes requires binding of Ino2-Ino4 transcription activator on the UAS_{INO} element. The transcription activation is repressed by a binding of Opi1 to Ino2. Under different growth conditions of yeasts such as; inositol starvation, zinc supplementation or exponential growth, where the PA level is high; Opi1 transcription repressor binds with Scs2 and PA at the ER/nuclear membrane, and stays inactive outside the nucleus. In conditions such as inositol supplementation, zinc depletion or stationary phase, in which the PA level is low, Opi1 translocate to the nucleus and binds with Ino2, to repress the UAS_{INO} containing genes transcription. On the other hand, Zap1 is activated under zinc limited conditions, and enhances transcription of genes such as *PIS1* and *PAH1*. A bold line shows genes with activated transcription.

Transcription of the genes encoding Phosphatidylinositol synthase, *Pis1*; and phosphatidic acid hydrolase, *Pah1* are controlled by a zinc-sensing and zinc-inducible transcriptional activator, *Zap1*. This activator binds on zinc responsive cis-acting element (UAS_{ZRE}) of their promoters to activate transcription of these genes. Under different growth conditions such as; inositol supplementation, zinc depletion or stationary phase, the PA level is reduced; thus *Zap1* becomes activated and up-regulates the transcription of *PIS1*, resulting in an increase in the PI level, as revealed by [Carman and Han, \(2007\)](#); [Henry, \(2012\)](#).

5. Future perspective

We discussed the genes that are involved in the TAG synthesis, storage and mobilization, which contributed in the increase or decrease of the PI level. This PI is a major component of the lipid bilayer. A recent study of [Phan *et al.*, \(2019\)](#) added that PI is

phosphorylated, and acts as a regulator of membrane trafficking, cytoskeleton interaction and signal transduction. Furthermore, previous work of [Fujita and Kinoshita, \(2012\)](#) reported that PI is a precursor of glycosyl-phosphatidylinositol (GPI), which is a conserved post-translational modification among the eukaryotes. In yeasts, [Yoko-o *et al.*, \(2013\)](#) highlighted that GPI-anchored proteins are major components of the mannan layer in the cell wall structure, which are involved in cell wall biogenesis and integrity. Several reports suggested there is an inverse correlation between TAG and PI. However, PA seems to have a key role in the control of TAG and PI levels. The balance between PI and TAG might affect membrane trafficking, cellular signaling and GPI biosynthesis. Further researches are thus required on the cellular level to clarify the impact of the genes required for biosynthesis of PI and TAG.

Conclusion

TAG and PI levels are inversely correlated. Deletion of any of the genes encoding the proteins involved in LD bud formation results in the decrease of TAG level, and a concomitant increase of the PI level.

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Conflict of interest

The authors of this review declare no conflict of interest.

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Ethical Approval

Non-applicable.

6. References

- Adeyo, O.; Horn, J.P.; Lee, S.K.; Binns, D.D.; Chandrahas, A.; Chapman, K.D. and Goodman, J.M. (2011). The Yeast Lipin Orthologue Pah1p Is Important for Biogenesis of Lipid Droplets. *The Journal of Cell Biology*. 192(6): 1043-1055.
- Athenstaedt, K. and Daum, G. (2005). Tgl4p and Tgl5p, Two Triacylglycerol Lipases of the Yeast *Saccharomyces cerevisiae* Are Localized to Lipid Particles. *Journal of Biological Chemistry*. 280(45): 37301-37339.
- Bachhawat, N.; Ouyang, Q. and Henry, S.A. (1995). Functional Characterization of an Inositol-Sensitive Upstream Activation Sequence in Yeast. *Journal of Biological Chemistry*. 270(42): 25087-25095.
- Barbosa, A.D.; Sembongi, H.; Su, W.; Abreu, S.; Reggiori, F.; Carman M.G. and Siniossoglou, S. (2015). Lipid Partitioning at the Nuclear Envelope Controls Membrane Biogenesis. *Molecular Biology of the Cell*. 26(20): 3641-3657.
- Brasaemle, D.L. and Wolins, N.E. (2012). Packaging of Fat: An Evolving Model of Lipid Droplet Assembly and Expansion. *Journal of Biological Chemistry*. 287(4): 2273-2279.
- Carman, G.C. and Han, S.G. (2018). Phosphatidate Phosphatase Regulates Membrane Phospholipid Synthesis via Phosphatidylserine Synthase. *Advances in Biological Regulation*. 67: 49-58.
- Carman, G.M. and Han, S.G. (2007). Regulation of Phospholipid Synthesis in *Saccharomyces cerevisiae* by Zinc Depletion. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*. 1771(3): 322-330.
- Chen, X. and Goodman, M.J. (2017). The Collaborative Work of Droplet Assembly. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*. 1862 (10): 1205-1211.
- Choudhary, V.; Golani, G.; Joshi, S.A.; Schneider, R.; Prinz, A.W. and Kozlov, M.M. (2018). Architecture of Lipid Droplets in Endoplasmic Reticulum Is Determined by Phospholipid Intrinsic Curvature. *Current Biology*. 28(6): 915-926.
- Choudhary, V.; Ojha, N.; Golden, A. and Pinz, A.W. (2015). A Conserved Family of Proteins Facilitates Nascent Lipid Droplet Budding from the ER. *Journal of Cell Biology*. 211(2): 261-271.

- De Smet, C.H.; Vittone, E.; Scherer, Max.; Houweling, M.; Liebisch, G.; brouwers, J.F. and De Kroon, A.I.P.M. (2012).** The Yeast Acyltransferase Sct1p Regulates Fatty Acid Desaturation by Competing with the Desaturase Ole1p. *Molecular Biology of the Cell*. 23(7): 1146-1156.
- Dietz, M.; Heyken, T.W.; Hoppen, J.; Geburtig, S. and Schüller, J.H. (2003).** TFIIB and Subunits of the SAGA Complex Are Involved in Transcriptional Activation of Phospholipid Biosynthetic Genes by the Regulatory Protein Ino2 in the Yeast *Saccharomyces cerevisiae*. *Molecular Microbiology*. 48(4): 1119-1130.
- Fahy, E.; Cotter, D.; Sud, M. and Subramaniam, S. (2011).** Lipid Classification, Structures and Tools. *Biochimica et Biophysica Acta (BBA). Molecular and Cell Biology of Lipids*. 1811(11): 637-647.
- Fei, W.; Wang, H.; Fu, X.; Bielby, C. and Yang, H. (2009).** Conditions of Endoplasmic Reticulum Stress Stimulate Lipid Droplet Formation in *Saccharomyces cerevisiae*. *Biochemical Journal*. 424(1): 61-67.
- Fujita, M. and Kinoshita, T. (2012).** GPI-Anchor Remodeling: Potential Functions of GPI-Anchors in Intracellular Trafficking and Membrane Dynamics. *Biochimica et Biophysica Acta (BBA). Molecular and Cell Biology of Lipids*. 1821(8): 1050-1058.
- Gaigg, B.; Neergaard, F.B.T.; Schneiter, R.; Hansen, K.J.; Færgeman, J.N.; Jensen, A.N.; Andersen, R.J.; Friis, J.; Sandhoff, R.; Schrøder, D.H. and Knudsen, J. (2001).** Depletion of Acyl-Coenzyme A-Binding Protein Affects Sphingolipid Synthesis and Causes Vesicle Accumulation and Membrane Defects in *Saccharomyces cerevisiae*. *Molecular Biology of the Cell*. 12(4): 1147-1160.
- Gao, Q.; Binns, D.D.; Kinch, N.L.; Grishin, V.N.; Ortiz, N.; Chen, X. and Goodman, M.J. (2017).** Pet10p Is a Yeast Perilipin That Stabilizes Lipid Droplets and Promotes Their Assembly. *Journal of Cell Biology*. 216(10): 3199-3217.
- Gaspar, M.L.; Hofbauer, F.H.; Kohlwein, D.S. and Henry, A.S. (2011).** Coordination of Storage Lipid Synthesis and Membrane Biogenesis. *Journal of Biological Chemistry*. 286(3): 1696-1708.
- Graef, M. (2018).** Lipid Droplet-mediated Lipid and Protein Homeostasis in Budding Yeast. *FEBS Letters*. 592(8): 1291-1303.
- Gross, D.A.; Zhan, C. and Silver, L.D. (2011).** Direct Binding of Triglyceride to Fat Storage-Inducing Trans-membrane Proteins 1 and 2 Is Important for Lipid Droplet Formation. *Proceedings of the National Academy of Sciences*. 108(49): 19581-19586.
- Guo, Y.; Cordes, R.K.; Farese, V.R. and Walther, C.T. (2009).** Lipid Droplets at a Glance. *Journal of Cell Science*. 122(6): 749-752.
- Ham, H.J.; Rho, J.H.; Shin, K.S. and Yoon, J.H. (2010).** The TGL2 Gene of *Saccharomyces cerevisiae* Encodes an Active Acylglycerol Lipase Located in the Mitochondria. *Journal of Biological Chemistry*. 285(5): 3005-3013.
- Han, S.; Binns, D.D.; Chang, F.Y. and Goodman, M.J. (2015).** Dissecting Seipin Function: The Localized Accumulation of Phosphatidic Acid at ER/LD Junctions in the Absence of Seipin Is Suppressed by Sei1p Δ Nterm Only in Combination with Ldb16p. *BMC Cell Biology*. 16(1): 29.
- Han, G.S.; O'Hara, L.; Carman, M.G. and Siniossoglou, S. (2008).** An Unconventional Diacylglycerol Kinase That Regulates Phospholipid Synthesis and Nuclear Membrane Growth. *Journal of Biological Chemistry*. 283(29): 20433-20442.
- Hartman, I.Z.; Liu, P.; Zehmer, K.J.; Phelps, L.K.; Jo, Y.; Anderson, W.G.R. and DeBose-Boyd, A.R. (2010).** Sterol-Induced Dislocation of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase from Endoplasmic Reticulum Membranes into the Cytosol through a Subcellular Compartment Resembling Lipid Droplets. *Journal of Biological Chemistry*. 285(25): 19288-19298.

- Henry, S.A.; Kohlwein, D.S. and Carman, M.G. (2012).** Metabolism and Regulation of Glycerolipids in the Yeast *Saccharomyces cerevisiae*. *Genetics*. 190(2): 317-349.
- Jacquier, N.; Choudhary, V.; Mari, M.; Toulmay, A.; Reggiori, F. and Schneider, R. (2011).** Lipid Droplets Are Functionally Connected to the Endoplasmic Reticulum in *Saccharomyces cerevisiae*. *Journal of Cell Science*. 124(14): 2424-2437.
- Jain, S.; Stanford, N.; Bhagwat, N.; Seiler, B.; Costanzo, M.; Boone, C. and Oelkers, P. (2007).** Identification of a Novel Lysophospholipid Acyltransferase in *Saccharomyces cerevisiae*. *Journal of Biological Chemistry*. 282(42): 30562-30569.
- Kadereit, B.; Kumar, P.; Wang, W.J.; Miranda, D.; Snapp, L.E.; Severina, N.; Torregroza, I.; Evans, T. and Silver, L.D. (2008).** Evolutionarily Conserved Gene Family Important for Fat Storage. *Proceedings of the National Academy of Sciences*. 105(1): 94-99.
- Kimura, T.; Jennings, W. and Epanand, M.R. (2016).** Roles of Specific Lipid Species in the Cell and Their Molecular Mechanism. *Progress in Lipid Research*. 62: 75-92.
- Klein, I.; Klug, L.; Schmidt, C.; Zandl, M.; Korber, M.; Daum, G. and Athenstaed, K. (2016).** Regulation of the Yeast Triacylglycerol Lipases Tgl4p and Tgl5p by the Presence/Absence of Nonpolar Lipids. *Molecular Biology of the Cell*. 27(13): 2014-2024.
- Klug, L. and Daum, G. (2014).** Yeast Lipid Metabolism at a Glance. *FEMS Yeast Research*. 14(3): 369-388.
- Köffel, R.; Tiwari, R.; Falquet, L. and Schneider, R. (2005).** The *Saccharomyces cerevisiae* YLL012/YEH1, YLR020/YEH2, and TGL1 Genes Encode a Novel Family of Membrane-Anchored Lipases That Are Required for Steryl Ester Hydrolysis. *Molecular and Cellular Biology*. 25(5): 1655-1668.
- Krahmer, N.; Hilger, M.; Kory, N.; Wilfling, F.; Stoehr, G.; Mann, M.; Farese, V.R. and Walther, C.T. (2013).** Protein Correlation Profiles Identify Lipid Droplet Proteins with High Confidence. *Molecular and Cellular Proteomics*. 12(5): 1115-1126.
- Listenberger, L.L.; Han, X.; Lewis, E.S.; Cases, S.; Farese, V.R.; Ory, S.D. and Schaffer, E.J. (2003).** Triglyceride Accumulation Protects against Fatty Acid-Induced Lipotoxicity. *Proceedings of the National Academy of Sciences*. 100(6): 3077-3082.
- Loewen, C.J.R.; Gaspar, M.L.; Jesch, S.A.; Delon, A.; Ktistasis, N.T.; Henry, S.A. and Levine, T.P. (2004).** Phospholipid Metabolism Regulated by a Transcription Factor Sensing Phosphatidic Acid. *Science*. 304(5677): 1644-1647.
- Markgraf, D.F.; Klemm, W.R.; Junker, M.; Hannibal-Bach, K.H.; Ejsing, S.C. and Rapoport, A.T. (2014).** An ER Protein Functionally Couples Neutral Lipid Metabolism on Lipid Droplets to Membrane Lipid Synthesis in the ER. *Cell Reports*. 6(1): 44-45.
- Nettebrock, N.T. and Bohnert, M. (2020).** Born This Way – Biogenesis of Lipid Droplets from Specialized ER Subdomains. *Biochimica et Biophysica Acta (BBA). Molecular and Cell Biology of Lipids*. 1865(1): 158448.
- Olzmann, J.A. and Carvalho, P. (2019).** Dynamics and Functions of Lipid Droplets. *Nature Reviews Molecular Cell Biology*. 20(3): 137-155.
- Phan, T.K.; Williams, A.S; Bindra, K.G.; Lay, T.F.; Poon, K.H.I. and Hulett, D.M. (2019).** Phosphoinositides: Multipurpose Cellular Lipids with Emerging Roles in Cell Death. *Cell Death and Differentiation*. 26(5): 781-793.
- Ploegh, H.L. (2007).** A Lipid-Based Model for the Creation of an Escape Hatch from the Endoplasmic Reticulum. *Nature*. 448(7152): 435-438.
- Pol, A.; Gross, P.S. and Parton, G.R. (2014).** Biogenesis of the Multifunctional Lipid Droplet:

Lipids, Proteins, and Sites. *The Journal of Cell Biology*. 204(5): 635-646.

Qi, Y.; Kapterian, S.T.; Du, X.; Ma, Q.; Fei, W.; Zhang, Y.; Huang, X.; Dawes, W.I and Yang, H. (2016). CDP-Diacylglycerol Synthases Regulate the Growth of Lipid Droplets and Adipocyte Development. *Journal of Lipid Research*. 57(5): 767-780.

Rajakumari, S.; Rajasekharan, R. and Daum, G. (2010). Triacylglycerol Lipolysis Is Linked to Sphingolipid and Phospholipid Metabolism of the Yeast *Saccharomyces cerevisiae*. *Biochimica et Biophysica Acta (BBA). Molecular and Cell Biology of Lipids*. 1801(12): 1314-1322.

Riekhof, W.R.; Wu, J.; Jones, L.J. and Voelker, R.D. (2007). Identification and Characterization of the Major Lysophosphatidylethanolamine Acyltransferase in *Saccharomyces cerevisiae*. *Journal of Biological Chemistry*. 282(39): 28344-28352.

Schmidt, C.; Athenstaedt, K.; Koch, B.; Ploier, B. and Daum, G. (2013). Regulation of the Yeast Triacylglycerol Lipase Tgl3p by Formation of Nonpolar Lipids. *Journal of Biological Chemistry*. 288(27): 19939-19948.

Teixeira, V.; Johnsen, L.; Martinez-Montanes, F.; Grippa, A.; Buxo, L.; Idrissi, F.; Ejsing C.S. and Carvalho, P. (2018). Regulation of Lipid Droplets by Metabolically Controlled Ldo Isoforms. *Journal of Cell Biology*. 217(1): 127-138.

Thiam, A.R. and Forêt, L. (2016). The Physics of Lipid Droplet Nucleation, Growth and Budding. *Biochimica et Biophysica Acta (BBA). Molecular and Cell Biology of Lipids*. 1861(8): 715-722.

Van der Veen, J.N.; Kennely, J.P.; Wan, S.; Vance, J.E.; Vance, D.E. and Jacobs, R.L. (2017). The Critical Role of Phosphatidylcholine and Phosphatidylethanolamine Metabolism in Health and Disease. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 1859(9): 1558-1572.

Velázquez, A.P.; Tatsuta, T.; Ghillebert, R.; Drescher, I. and Graef, M. (2016). Lipid Droplet-Mediated ER Homeostasis Regulates Autophagy and Cell Survival during Starvation. *Journal of Cell Biology*. 212(6): 621-631.

Walther, T.C.; Chung, J. and Faresse jr, R.V. (2017). Lipid Droplet Biogenesis. *Annual Review of Cell and Developmental Biology*. 33(1): 491-510.

Wang, W.; Xin, J.; Yang, X.; Lam, S.M.; Shui, G.; Wang, Y. and Huang, X. (2019). Lipid-Gene Regulatory Network Reveals Coregulations of Triacylglycerol with Phosphatidylinositol/Lysophosphatidylinositol and with Hexosyl-Ceramide. *Biochimica et Biophysica Acta (BBA). Molecular and Cell Biology of Lipids*. 1864(2): 168-180.

Wang, S.; Idrissi, F.Z.; Hermansson, M.; Grippa, A.; Ejsing, C.S. and Carvalho, P. (2018). Seipin and the Membrane-Shaping Protein Pex30 Cooperate in Organelle Budding from the Endoplasmic Reticulum. *Nature Communications*. 9(1): 2939.

Wang, H.; Bcuwe, M.; Housden, B.E.; Chitraju, C.; Porras, A.J; Graham M.M.; Liu, X.N., et al. (2016). Seipin Is Required for Converting Nascent to Mature Lipid Droplets. *Elife*. 5: e16582.

Wilfling, F.; Haas, J.T.; Walther, T.C. and Faresse, R.V. (2014). Lipid Droplet Biogenesis. *Current Opinion in Cell Biology*. 29(8): 39-45.

Yeagle, P.L. (2016). Lipid-Protein Interactions in Membranes. In *The Membranes of Cells*. 291-334.

Yoko-o., T.; Ichikawa, D.; Miyagishi, Y.; Kato, A.; Umemura, M.; Takase, K.; Ra, M.; Ikeda, K.; Taguchi, R. and Jigami, Y. (2013). Determination and Physiological Roles of the Glycosylphosphatidylinositol Lipid Remodelling Pathway in Yeast. *Molecular Microbiology*. 88(1): 140-155.

Zheng, Z. and Zou, J. (2001). The Initial Step of the Glycerolipid Pathway. *Journal of Biological Chemistry*. 276(45): 41710-41716.