Univerzita Karlova

Přírodovědecká fakulta

Studijní program: Speciální chemicko-biologické obory

Studijní obor: Molekulární biologie a biochemie organismů

Michaela Grulyová

Propojení mezi metabolismem dusíku, signální dráhou TOR a metabolismem lipidů
Crosstalks between nitrogen metabolism, the TOR pathway and the metabolism of lipids

Typ závěrečné práce:

Bakalářská práce

Vedoucí práce/Školitel: RNDr. Martin Převorovský, Ph.D.

Praha, 2021

Poděkování

Ráda bych poděkovala především svému školiteli Martinu Převorovskému za jeho vedení, vstřícnost a rady při psaní této práce.

Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracoval/a samostatně a že jsem uvedl/a všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 6.5.2021

Podpis

Table of contents

2. List of abbreviations 1
3. Introduction 2
4. The TOR pathway 2
5. Lipid accumulation 5
5.1. Lipid accumulation caused by rapamycin 6
5.1.1. Lipid accumulation in unicellular organisms 7
5.1.2 Lipid accumulation in mammals 8
5.2. Lipid accumulation upon nitrogen depletion 8
5.3. Lipid accumulation caused by TORC1 activation 9
5.4. Lipid accumulation caused by TORC2 inhibition 10
6. Decrease in lipid content 11
6.1. Decrease in lipid content caused by nitrogen replenishment 11
6.2. Decrease in lipid content caused by TORC1 inhibition 11
7. Adipocyte differentiation 12
8. Summary 12
9. Conclusion 14

1. Abstract

Cells coordinate their metabolism based on various factors, for example nitrogen availability. The TOR pathway is an important regulator of nitrogen metabolism, it has a role in sensing intracellular amino acids status, and it controls especially cell growth, protein synthesis, proliferation and cell survival. However, it has been shown that the TOR pathway also controls lipid biosynthesis and lipid accumulation through various mechanisms in response to nitrogen availability. Although the TOR pathway is well conserved among the eukaryotic organisms, its outcomes differ diametrically when it comes to the lipid accumulation. This essay provides some insides into the mechanisms of regulation of the lipid metabolism by the TOR pathway.

Keywords: nitrogen metabolism, lipid metabolism, TOR pathway, eukaryotic organisms, rapamycin, lipid accumulation

Abstrakt

Buňky musí koordinovat množství různých signálů, například dostupnost dusíku, a na základě toho uzpůsobovat svůj metabolismus. Signální dráha TOR patří mezi důležité regulační mechanismy metabolismu dusíku. Na základě intracelulární dostupnosti aminokyselin řídí především buněčný růst, syntézu proteinů, proliferaci a přežití buňky. Dále bylo zjištěno, že různými mechanismy řídí také biosyntézu a akumulaci lipidů, a to v reakci na dostupnost dusíku. Přestože je dráha TOR u eukaryotických organismů dobře konzervovaná, účinky její regulace se diametrálně liší, co se akumulace lipidů týče. Tato práce představuje některé mechanismy, kterými dráha TOR reguluje metabolismus lipidů.

Klíčová slova: metabolismus dusíku, metabolismus lipidů, dráha TOR, eukaryotické organismy, rapamycin, akumulace lipidů

2. List of abbreviations

FFA	free fatty acid
LD	lipid droplets
LDL	low density lipoprotein
NCR	nitrogen catabolite repression
SE	sterol esters
SPS sensor system	Ssy1-Ptr3-Ssy5 signalling sensor system
TAG	triacylglycerols
VLDL	very low density lipoprotein

3. Introduction

For the survival of the cell it is necessary to manage and coordinate numerous signals, for example the nitrogen availability. Nitrogen metabolism is fundamental for supplying energy metabolism and producing cellular components such as proteins, amino acids, nucleotides and other nitrogenous compounds, thus it must be precisely regulated. Pathways involved in the nitrogen metabolism coordinate various catabolic and anabolic processes. Among these pathways belong the Ssy1-Ptr3-Ssy5 signalling sensor system (SPS sensor system), the target of rapamycin (TOR) regulatory pathway, nitrogen catabolite repression (NCR), and the general amino acid control (GAAC) pathway. NCR controls utilization of preferable nitrogen sources, while the TOR pathway is responsible for sensing intracellular amino acids (Zhang et al., 2018)

Along with establishing cellular homeostasis, lipids also have important structural, signalling and biosynthetic roles in an organism (Muro et al., 2014). Lipid metabolism is dependent on the availability of nutrients, such as carbohydrates, phosphorus, and amino acids. Based on that, cell either upregulates lipid biosynthesis and begins to accumulate lipids or shifts to catabolic processes like lipolysis and beta-oxidation. Storage of lipids according to nutrient availability is a key mechanism for survival of an organism during starvation. Lipids are accumulated either inside cell, or in blood plasma (Caron et al., 2015; Dzurendova et al., 2020; Saponaro et al., 2015).

It is becoming increasingly clear, that the TOR pathway plays an important intermediate between the nitrogen and the lipid metabolism. Based on the nitrogen levels, the TOR pathway regulates lipid metabolism through various mechanisms (Laplante et al., 2009). The TOR pathway is also associated with many diseases, such as obesity, type 2 diabetes, cancer, and neurological diseases, so it is extremely important to study its effects (Mao & Zhang, 2018).

4. The TOR pathway

The target of rapamycin is a highly conserved serine/threonine kinase and part of the phosphatidylinositol 3-kinase-related kinases family, with multiple regulatory functions in cell cycle and metabolism. The TOR pathway regulates proliferation, growth, autophagy and survival of the eukaryotic cell. In favourable conditions is the TOR pathway activated and promotes cell growth. There are two TOR complexes – TOR complex 1 (TORC1) and TOR complex 2 (TORC2) (Caron et al., 2015; Laplante et al., 2009). The composition of these complexes differs between organisms, containing some homologous and some organism-specific proteins (Cybulski & Hall, 2009a; Loewith & Hall, 2011). *Saccharomyces cerevisiae*

TORC1 consists of TOR, Lst8, Kog1 and yeast-specific Tco89. Mechanistic/mammalian TORC1 consists of mechanistic TOR (mTOR), mLST8, RAPTOR and vertebrate-specific DEPTOR. TOR is a homologue of mTOR, Lst8 is homologous with mLst8 and Kog1 with RAPTOR. *S. cerevisiae* TORC2 comprises TOR, Lst8, Avo3, Avo1, Bit61, Bit2, yeast-specific Avo2; mTORC2 comprises mTOR, mLST8, RICTOR, mSIN1, PROTOR-1, PROTOR-2 and DEPTOR. Yeast Avo3 is a homologue of RICTOR, Avo1 is a homologue of mSIN1, Bit61 and Bit2 are homologous with PROTOR-1 AND PROTOR-2. Both of the TOR complexes share some common parts - TOR, Lst8 and in the case of vertebrates also DEPTOR (Gaubitz et al., 2016). Budding and fission yeast have two TOR paralogs, Tor1 and Tor2. Both can be part of TORC1 but only Tor2 is involved in TORC2 in budding yeast. In fission yeast only Tor1 is part of TORC2, meaning that budding yeast Tor2 and fission yeast Tor1 are equivalent in their functions (Cybulski & Hall, 2009a).

In *S. cerevisiae* TORC1 is localized on the vacuolar membrane and is regulated in response to carbon, nitrogen and phosphate availability and also to stress conditions. Among the major upstream TORC1 regulators is the EGO complex, which is a protein complex sensing amino acid levels. TORC1 also regulates itself by feedback loops. The major downstream effector branches of TORC1 signalling are Sch9 and Tap42-PP2A pathways. These two pathways control protein synthesis, cell size, cell cycle progression, nutrient uptake, cell-wall integrity pathway, life span and autophagy (Loewith & Hall, 2011).

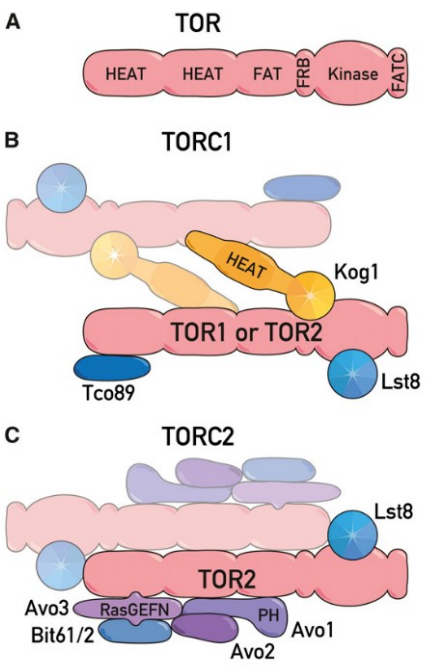

Figure 1: (A) Conserved domain structure of TOR from *S. cerevisiae*. (B) Composition of TOR complex 1. TORC1 is ~2 MDa in size and contains Kog1, Tco89, Lst8, and either TOR1 or TOR2. (C) Composition of TOR complex 2. TORC2 is ~2 MDa in size and contains Avo1, Avo2, Avo3, Bit61, and/or its paralog Bit2, Lst8, and TOR2 but not TOR1 (taken from Loewith & Hall, 2011).

Budding yeast TORC2 can be found near or at the plasma membrane and probably other unspecified subcellular localizations. One of its major substrates seems to be Nip7, a ribosome maturation factor. TORC2 is also controlled directly by the ribosome and inhibited by environmental stress. Another major TORC2 substrate appears to be Ypk protein kinase responsible for polarization of the actin cytoskeleton and sphingolipid biosynthesis (Loewith & Hall, 2011).

mTORC1 senses growth factors, energy status, oxygen and amino acids, and acts to regulate many processes involved in the control of cell growth and metabolism (Laplante et al., 2009). mTORC1 is affected by growth factors such as insulin and insulin-like growth factor through phosphoinositide 3-kinase (PI3K)-AKT-Tuberous sclerosis (TSC)-RHEB signalling. Amino acids activate mTORC1 signalling through the Rag family of GTPases, which promotes mTORC1 translocation to the lysosome, where it is activated by RHEB. Stress such as hypoxia, DNA damage also influence mTORC1 through TSC1/2. Activated mTORC1 induces protein synthesis via its major downstream effectors the ribosomal S6 kinase (S6K) and the inhibitory eIF4E-binding proteins (4E-BPs), which subsequently promote translation initiation and elongation through ribosomal protein S6 (S6K1), protein synthesis initiation factor 4B (eIF4B), and elongation factor 2 kinase (eEF2K) (Mao & Zhang, 2018).

mTORC2 responds to growth factors and regulates cell survival, cell metabolism, and cytoskeletal organization (Laplante et al., 2009) mTORC2 is activated by the growth factors such as insulin and IGF too, but is insensitive to the nutrients. Activated mTORC2 phosphorylates AKT from AGC kinase family, AKT phosphorylates TSC2, the upstream inhibitor of mTORC1, so that activation of mTORC2 inactivates mTORC1. It also works the other way around, mTORC1 can phosphorylate mSIN1 via S6K axis and inactivate mTORC2 (Mao & Zhang, 2018).

Besides other TORC functions, it also seems to regulate autophagy and surprisingly lipid metabolism. TORC1 inhibits autophagy by direct phosphorylation of Atg13, which is required for the induction of macroautophagy (Loewith & Hall, 2011).

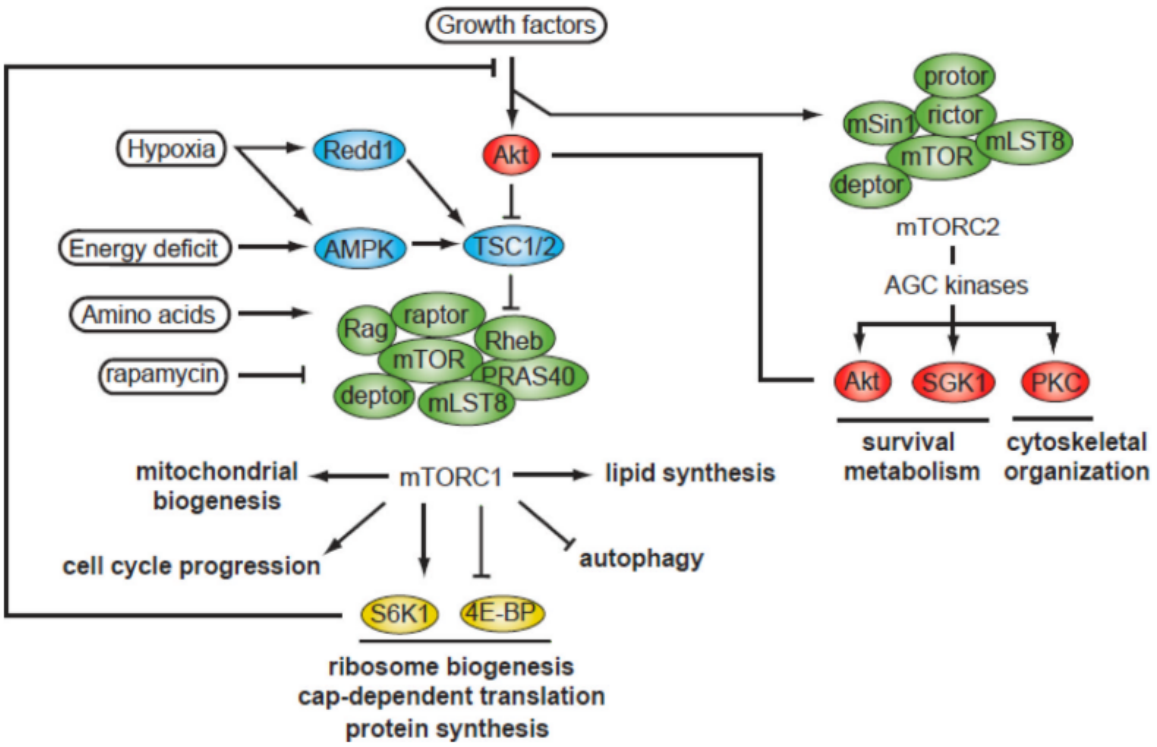

Figure 2: Overview of the mTOR signalling pathway. The mTOR kinase nucleates two characterized protein complexes termed mTORC1 and mTORC2 (taken from Laplante et al., 2009).

5. Lipid accumulation

Most cells are able to store neutral lipids in their cytoplasm in the form of dynamic organelles called lipid droplets or also lipid bodies or lipid particles. Lipid droplets (LD) consist of a phospholipid monolayer surrounding its hydrophobic core, creating a barrier to delimit it from the aqueous environment of the cytosol. The hydrophobic core contains mainly sterol esters (SE) and triacylglycerols (TAG); the ratio between SE and TAG depends on the cell type. Besides SE and TAG, retinyl esters, wax esters, ether lipids and long-chain isoprenoids can be found in LDs (Walther & Farese, 2012). LD's foremost function is to store energy and building blocks for membrane assembly, but it also has other important functions such as lipid synthesis, protection from lipotoxicity, protein storage, inflammatory response and viral replication (Bozza & Viola, 2010; Walther & Farese, 2012). These other functions are allowed by proteins associated with LDs, such as DGAT2, diacylglycerol O-Acyltransferase 2, which participates in TAG synthesis. LDs are also interesting as a possible resource for biodiesel production and because of their linkage to some diseases, such as cancer, diabetes and obesity (Walther & Farese, 2012).

TAGs are synthetised from free fatty acids (FFA) and glycerol-3-phosphate. TAG synthesis occurs principally in the adipose tissue, but also in the liver, muscle, heart and pancreas. FFAs for TAG synthesis derive either from the diet, peripheral lipolysis or de novo lipogenesis. De novo lipogenesis appears mostly in the livers after carbohydrate intake. Lipogenesis is controlled by hormones including insulin that stimulates lipid synthesis and adipogenesis (Saponaro et al., 2015).

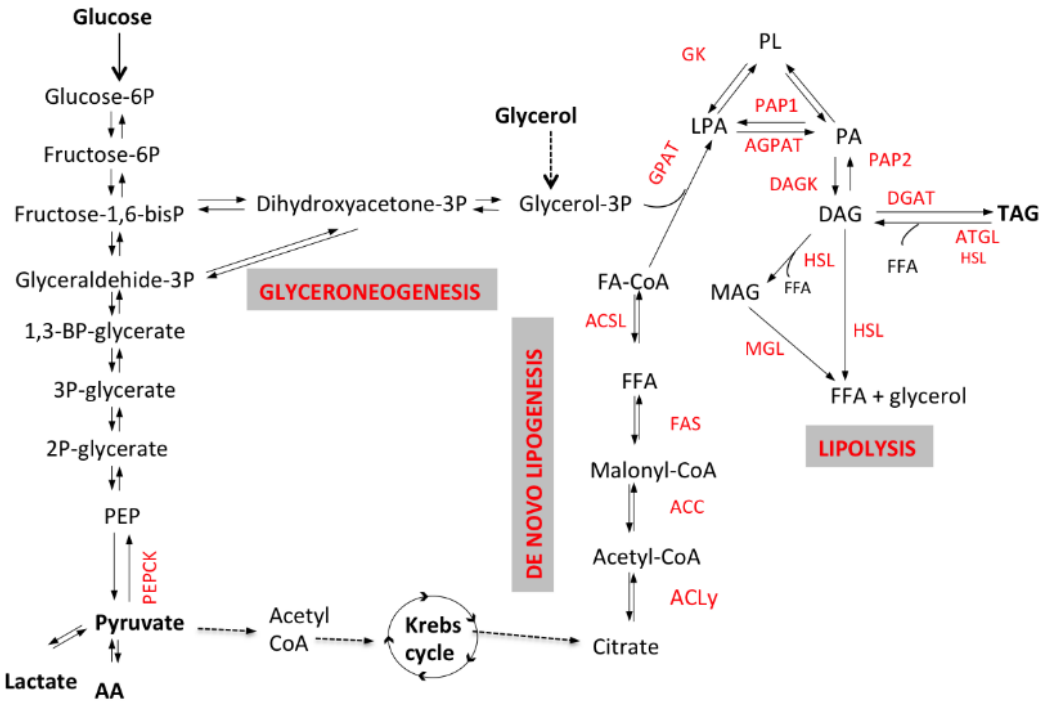

Figure 3: Schematic representation of lipolytic and lipogenic pathways (taken from Saponaro et al., 2015).

5.1. Lipid accumulation caused by rapamycin

Rapamycin is a specific antifungal inhibitor produced by the bacteria *Streptomyces hygroscopicus*, which binds to its intracellular receptor FKBP12 and directly inhibits the kinase activity of TORC1 (Caron et al., 2015). However, in some mammalian cell types rapamycin also seems to inhibit TORC2 upon long-term application because it prevents TORC2 assembly (Cybulski & Hall, 2009b). Treatment with rapamycin shows some symptoms similar to nutrient starvation, such as G0 arrest, growth inhibition, induction of autophagy and decrease in protein synthesis. Rapamycin also has immunosuppressive effects, which is why it is used preventively for transplant patients. Rapamycin is also used as a treatment of advanced kidney cancer (Loewith & Hall, 2011).

5.1.1. Lipid accumulation in unicellular organisms

Saccharomyces cerevisiae cells treated with rapamycin accumulate LDs by activating lipogenic phase, blocking lipolysis, and synthesising TAG but not SE. This effect is mediated by the Tap42-PP2A effector branch of TORC1 (Madeira et al., 2015). Activated TORC1 phosphorylates and binds Tap42 in complexes with PP2A and Sit4, which is a PP2A-like protein phosphatase. These complexes are located at the endosomal/vacuolar membrane and in this state are inactive. Upon rapamycin treatment or starvation, the complexes are released to the cytoplasm, where they act to dephosphorylate transcription factors Gln3 and Gat1. Gln3 and Gat1 are then relocated from the cytoplasm to the nucleus. Gln3 and Gat1 participate as transcriptional activators of genes for usage of poor nitrogen sources in nitrogen catabolite repression, a pathway regulating utilization of nitrogen sources (Broach, 2012). Rapamycin treatment does not affect LD accumulation in *gln3*Δ and *gat1*Δ strains. In *sit4*Δ strain the LD accumulation caused by rapamycin is partially suppressed (Madeira et al., 2015).

It was further found, that *Saccharomyces cerevisiae* TORC1 also impacts on Rtg1 and Rtg3 transcription factors, members of the retrograde pathway (Komeili et al., 2000). The retrograde pathway is involved in transcriptional changes which are driven by the mitochondria. Mitochondrial dysfunctions are signalled to the nucleus to induce an appropriate transcriptional response. Mitochondria produces amino acid precursors, so the mitochondrial dysfunction is signalled by changes in glutamate or glutamine levels. This signal indirectly affects the localization of Rtg1 and Rtg3, which are allowed to the nucleus (Loewith & Hall, 2011). Rtg1 and Rtg3 are relocated into the nucleus as a result of rapamycin addition (Komeili et al., 2000). LD accumulation caused by rapamycin is not induced in *rtg1*Δ and *rtg3*Δ strains (Madeira et al., 2015).

Another protein important for lipid metabolism is Pah1, a yeast lipin homologue (Csaki & Reue, 2010), which encodes phosphatidic acid phosphohydrolase 1. Pah1 is important to generate diacylglycerol for the TAG synthesis (Olzmann & Carvalho, 2019), and for the biogenesis of LDs (Adeyo et al., 2011). Fission yeast TORC1 inhibition by rapamycin does not induce TAG accumulation in *pah1*Δ strain. TORC1 controls Pah1 via the Nem1-Spo7 phosphatase complex (Dubots et al., 2014).

Acyl-CoA:diacylglycerol acyltransferase Dga1 is also necessary for the TAG accumulation induced by rapamycin in yeast (Dubots et al., 2014; Madeira et al., 2015). Dga1 is responsible for the final step of TAG synthesis (Walther & Farese, 2012).

5.1.2 Lipid accumulation in mammals

Rapamycin was found to cause lipid accumulation not only in unicellular organism, but also in mammals. Rapamycin causes hyperlipidemia in kidney transplant recipients and in animal models (Aggarwal et al., 2006; Lopes et al., 2014; Morrisett et al., 2003). In guinea pigs treated with rapamycin an increase in the concentration of plasma TAG, FFA, as well as increased TAG and cholesterol in the aorta were observed. In agreement with that, very low density lipoprotein (VLDL) and low density lipoprotein (LDL) particles isolated from these guinea pigs were bigger in size than the particles from non-treated animals (Aggarwal et al., 2006). The VLDL and LDL particles contain TAG circulating in the blood plasma (Saponaro et al., 2015). The increase in size was caused by the fact that the VLDL and LDL particles carried more TAG molecules (Aggarwal et al., 2006). Upon rapamycin treatment rats increase lipolysis in adipose tissue and decrease adipose storage, but ectopically accumulate lipids in muscle and liver (Lopes et al., 2014).

5.2. Lipid accumulation upon nitrogen depletion

The fatty acid profile under the nitrogen limitation is similar to that caused by rapamycin treatment, so it is not very surprising that lipid accumulation occurs too. However, the increase in the lipid accumulation under the nitrogen limited conditions is comparatively bigger and a reduction in growth occurs (Bracharz et al., 2017). The lower the concentration of nitrogen in the medium is, the more lipids are accumulated by the oleaginous yeast (Han et al., 2016).

Microalgae *Symbiodinium* and *Chlamydomonas reinhardtii* were also proved to accumulate lipids in nitrogen limited environment, specifically TAGs and SEs in the form of LDs. After re-addition of nitrogen, the level of accumulated lipids decreases. One of the protein kinases regulating lipid metabolism and accumulation is DYRKP. DYRKP works as a negative regulator of lipid accumulation in green microalgae (Jiang et al., 2014; Roustan et al., 2017; Schulz-Raffelt et al., 2016). *DGAT1*, *DGTT1* and *PDAT1* genes encoding acyltransferases, responsible for TAG biosynthesis, show increased expression in nitrogen deprived conditions. (Boyle et al., 2012; Schmollinger et al., 2014; Walther & Farese, 2012). Active TORC1 mediates phosphorylation of its downstream substrate EIF4B, a protein synthesis initiation factor (Mao & Zhang, 2018). Thus, the decreasing phosphorylation level of EIF4B during nitrogen depletion is a sign of TOR inactivation. However, Atg13 phosphorylation was increasing during nitrogen depletion (Roustan et al., 2017). Atg13 is a direct phosphorylation

target of TORC1 and part of the Atg1 complex, which is essential for the induction of autophagy (Broach, 2012). These data suggest a more complicated regulatory mechanism, possibly involving the PP2A branch of TORC1 signalling (Roustan et al., 2017).

5.3. Lipid accumulation caused by TORC1 activation

Surprisingly, not only mTORC1 inhibition, but also its activation promotes lipid accumulation, specifically de novo lipogenesis, intracellular accumulation of TAG, and suppresses lipolysis. mTORC1 controls lipolysis by suppressing the expression of ATGL and HSL lipases (Partha Chakrabarti et al., 2010), which are involved in the hydrolyzation of fatty acids. ATGL, adipose triglyceride lipase, catalyses the initial step of lipolysis, converting triglycerides to diacylglycerols, while HSL, hormone sensitive lipase, catalyses the second step of lipolysis, which is conversion of diacylglycerols to triacylglycerols (Caron et al., 2015; Walther & Farese, 2012). mTORC1 control of ATGL is mediated via Egr1, early growth response protein 1. Egr1 directly inhibits the expression of ATGL (P. Chakrabarti et al., 2013).

Pigs exposed to a high concentration of atmospheric ammonia accumulate lipids in skeletal muscle. Genes involved in lipid synthesis and uptake were up-regulated, while genes involved in lipolysis and beta-oxidation were downregulated. Ammonia exposure leads to synthesis of branched chain amino acids and aromatic amino acids, which leads to mTOR activation (Caron et al., 2015; Tang et al., 2020).

One way of how TORC1 promotes lipid accumulation is through SREBPs. Sterol regulatory element-binding proteins is a family of transcriptional factors that controls expression of lipogenic genes and therefore lipid homeostasis (Caron et al., 2015). Another protein involved in lipid metabolism is Lipin 1, which is a phosphatidic acid phosphatase and participates in TAG synthesis (Csaki & Reue, 2010). mTORC1 phosphorylates lipin 1 and controls its localization in the cell (Peterson et al., 2011). When mTORC1 is active, lipin 1 is phosphorylated and stays in the cytoplasm. When mTORC1 is inhibited, lipin 1 localizes to the nucleus and blocks transcriptional activity of SREBP (Peterson et al., 2011). This way mTORC1 positively controls transcription of genes involved in de novo lipogenesis and sterol biosynthesis (Caron et al., 2015; Peterson et al., 2011).

SREBP1, member of the SREBP family, is a master transcription regulator of de novo lipogenesis. S6K1 is a kinase directly phosphorylated by mTORC1 and it is also involved in protein synthesis (Caron et al., 2015) TORC1 phosphorylates S6K1, which promotes the processing of SREBP1 through an unknown mechanism (Düvel et al., 2010).

Another way of lipid accumulation by TORC1 is via the Akt/mTOR pathway, with ribosomal protein S6 as the major downstream target of mTORC1 (Calvisi et al., 2011). Protein kinase B (Akt) is activated by insulin via its cell surface receptor PI3K. When active, Akt activates mTORC1 by inhibiting proline-rich Akt substrate 40kDa (PRAS40) and tuberous sclerosis 1/2 (TSC1/2), which are both negative regulators of mTORC1 (Bakan & Laplante, 2012). S6K1 is part of the 40S subunit of eukaryotic ribosomes and it upregulates protein synthesis (Mok et al., 2013). The Akt/mTORC1 pathway controls lipogenesis via both transcriptional and post-transcriptional mechanisms. Akt suppresses ubiquitination of SREBPs and thus decreases their proteasome degradation (Calvisi et al., 2011). Silencing of SREBPs blocks Akt-dependent lipogenesis and active TORC1 is necessary for SREBP1 nuclear accumulation (Porstmann et al., 2008).

Another example is that peripheral nervous system myelination is controlled by the mTORC1-RXRY-SRBP axis. Nuclear retinoid X receptor Y (RXRY) is controlled by mTORC1 and activates the promoter of SREBP1. Active mTORC1 is required for proper lipid biosynthesis in Schwann cells (Norrmén et al., 2014).

In macrophages, leptin triggers biosynthesis of LDs (Maya-Monteiro et al., 2008). Leptin is a proinflammatory cytokine produced by adipocytes and is involved in the regulation of food intake (Saponaro et al., 2015). LD accumulation triggered by leptin is mediated by the activation of the PI3K/mTOR pathway. Activated mTORC1 phosphorylates its downstream targets S6K1 and 4EBP, which activate the translation initiation (Maya-Monteiro et al., 2008). Phosphorylated 4EBP promotes protein synthesis when TORC1 is activated by growth factors or amino acids (Laplante et al., 2009).

5.4. Lipid accumulation caused by TORC2 inhibition

Not only TORC1 inhibition, but also TORC2 inhibition causes lipid accumulation. Mutations in Rictor, a component of TORC2, cause an increase in accumulated body fat in *Caenorhabditis elegans*. These mutations are thought to be loss-of-function mutations. Besides higher TAG accumulation, *rictor* mutants are smaller in body size, developmentally delayed and have short life span. In adult animals, *Rictor* is mostly expressed in head neurons and intestine. TORC2 regulates accumulation of fat through SGK1 (Jones et al., 2009) and AKT kinases (Soukas et al., 2009). Both AKT and SGK1, serum- and gluco-corticoid-induced protein kinase 1 , are members of the ACG kinase family and downstream effectors of TORC2 (Laplante et al., 2009).

6. Decrease in lipid content

Decreasing lipid content is connected to lipolysis and β-oxidation. Lipolysis is a catabolic pathway, that comprises the hydrolysis of TAG by lipases into fatty acids and glycerol. Fatty acids and glycerol are eventually released into the plasma to circulate. Excess lipolysis can lead to high free FFA flux into the liver and subsequently lipotoxicity. β-oxidation is a pathway that produces energy from the TAG hydrolysis for homeostasis of cells and tissues. It takes place in the mitochondria and produces acetyl-CoA. The oxidation of fatty acids is elevated during fasting and starvation (Saponaro et al., 2015).

6.1. Decrease in lipid content caused by nitrogen replenishment

Induced lipid accumulation is completely reversible after nitrogen replenishment in green microalgae. This effect is caused by quick activation of β-oxidation pathways (Roustan et al., 2017; Valledor et al., 2014). In yeast, genes involved in lipid biosynthesis are downregulated after nitrogen repletion (Tesnière et al., 2019).

6.2. Decrease in lipid content caused by TORC1 inhibition

Quite controversially, TORC1 inhibition can cause both lipid accumulation and its decrease. In mammals, activation of de novo lipid synthesis requires mTORC1 function. Inhibition of mTORC1 blocks accumulation of fatty acids as well as phosphatidylcholine and phosphatidylglycerol levels. Both de novo lipid synthesis and accumulation of fatty acids are dependent on Akt activity (Porstmann et al., 2008). Rapamycin treatment and amino acid deprivation upregulate genes involved in fatty acids oxidation and downregulate genes involved in de novo biosynthesis of lipids (Peng et al., 2002).

Increased β-oxidation both in vivo and in vitro in skeletal muscle cells corresponds with elevated activity of CPT I and CPT II (Sipula et al., 2006). CPT I, carnitine palmitoyl transferase I, catalyses the transfer of the acyl group of a long-chain fatty acyl-CoA to carnitine to form acylcarnitine, this step enables acyl-CoA to enter the mitochondria. CPT II, carnitine palmitoyl transferase II, reconverts acylcarnitine in acyl-CoA and carnitine, then acyl-CoA enters β-oxidation (Saponaro et al., 2015). Elevated level of lipolysis is also caused by the increased expression of ATGL in both white and brown adipose tissue in vitro, while the expression of HSL is not affected by rapamycin treatment (Partha Chakrabarti et al., 2010).

Similar effect was also observed in the primary cultures of rat hepatocytes. Cells treated with rapamycin increased oxidation of exogenous fatty acids, esterification of exogenous fatty acids and reduced de novo lipid synthesis. There was a significant decrease in the expression of ACC (Brown et al., 2007). ACC, acetyl-CoA carboxylase, converts acetyl-CoA to malonyl-CoA. This is one of the steps in de novo lipogenesis (Saponaro et al., 2015). Expression of glycerol phosphate acyltransferase, GPAT, was also decreased (Brown et al., 2007). GPAT is involved in TAG synthesis (Saponaro et al., 2015).

7. Adipocyte differentiation

Adipogenesis consists of two phases. During the first phase called determination, cells commit to differentiation from multipotent mesenchymal stem cells to pre-adipocytes. The second phase, known as terminal differentiation, includes transfer of the pre-adipocytes to mature adipocytes. Adipogenesis is driven by a temporally regulated set of gene-expression events. Peroxisome proliferator-activated receptor γ, PPARγ, is a crucial transcription factor, its expression is necessary and sufficient for adipogenesis. SREBP1c functions as a pro-adipogenic transcription factor that induces PPARγ expression. CCAAT/enhancer-binding protein-α, C/EBPα, is another transcription factor, that directly induces many adipocyte genes. (Rosen & MacDougald, 2006).

Rapamycin inhibits adipocyte differentiation by decreasing the adipogenesis and lipogenesis. Rapamycin treatment downregulates expression of most adipocyte marker genes, such as PPARγ and SREBP1c. It was also found that constitutive mTOR activation is necessary for adipogenesis (Bell et al., 2000; Cho et al., 2004).

The precise mechanism of the adipogenesis inhibition by rapamycin is that it directly targets the transactivation activity of PPARγ, which is necessary for the positive transcriptional feedback control of C/EBPα expression and the adipogenic gene expression program. PPAR-γ activity is also dependent on amino acid sufficiency (Kim & Chen, 2004; Rosen & MacDougald, 2006).

8. Summary

Pathways of nitrogen metabolism influences the lipid metabolism based on nitrogen intake, mainly through the TOR pathway. The connection between nitrogen and lipid metabolism is regulated through various mechanisms, for example via Kes1, an oxysterol-binding protein (Caron et al., 2015; Mousley et al., 2012). It has been also discovered that not

only the nitrogen availability, but also its form determines lipid droplet formation in fission yeast (Zach et al., 2018).

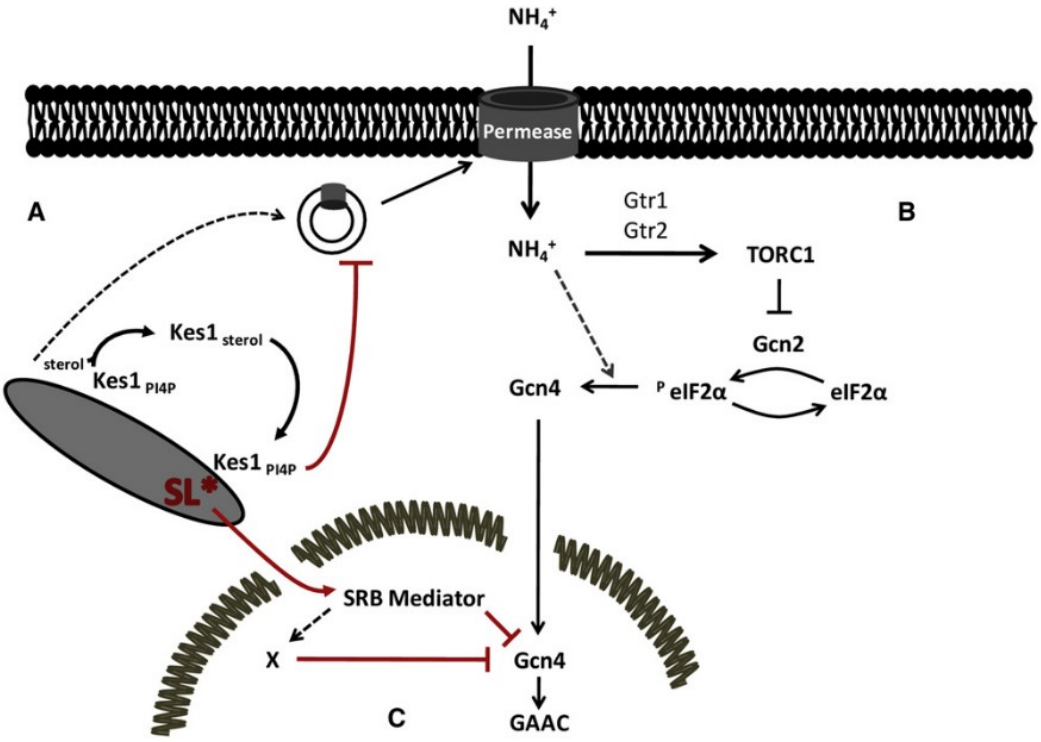

Figure 4: Proposed scheme of integration between lipid and nitrogen mechanism mediated by Kes1. (A) Membrane trafficking through a signalling TGN/endosomal compartment is defined by Sec14-regulated production of PtdIns-4-P countered by Kes1- mediated PtdIns-4-P sequestration. Degree of sequestration is set by PtdIns-4-P-mediated recruitment of Kes1 to TGN/endosomal membranes, and released by binding of the membrane-bound Kes1 to active sterol. (B) Kes1-mediated trafficking defects reduce efficiency of amino acid and NH4+ permease delivery to the plasma membrane. The incipient nitrogen stress results in reduced TOR activity and engagement of the early stages of the GAAC (phosphorylation of eIF2a). Efficiency of the terminal stages of the GAAC is determined by potency of Gcn4 antagonism levied by activity of the large SRB Mediator complex. (C) Potency of a Kes1-mediated trafficking arrest/delay sets the amplitude of TGN/endosomal production of SL available for nuclear signalling. SL* denotes a sphingolipid with signalling power (e.g., ceramide) produced in TGN/endosomal compartments. The potency of SL* signalling is directly proportional to Kes1 activity, and inhibits transcription initiation/elongation of genes loaded with Gcn4 and RNA polymerase II via action of the CDK8 module of the large SRB Mediator complex. SL* signalling foils the GAAC and induces cells to enter a metabolically coherent quiescence with properties of post mitotic cell physiology (taken from Mousley et al., 2012).

Although the TOR pathway is quite well conserved among the eukaryotic organisms, its effects on lipid metabolism differ a lot. TORC1 inhibition causes lipid accumulation in the unicellular organisms, but in the cells of multicellular organisms, it causes lipid reduction (Laplante et al., 2009; Madeira et al., 2015). In theory, this could be because, unlike the multicellular organisms, unicellular organisms can not rely on other cells, so they need to

accumulate storage lipids in the times of nitrogen deficiency. This would be in agreement with the fact, that *S. cerevisiae* cells consume glycogen in proper growth conditions to grow rapidly, but store glycogen during nutrient limitation (François & Parrou, 2001; Madeira et al., 2015).

Another explanation could be that LDs in mammals have besides its common role as lipid storage also some extra roles, such as immune response and viral replication (Madeira et al., 2015; Walther & Farese, 2012).

However, even more striking is the fact, that lipid accumulation is caused by both activation and inhibition of mTORC1 in mammals. The inhibition of TORC1 causes hyperlipidemia in long-term usage, but in skeletal muscle, adipose tissue and hepatocytes under the rapamycin treatment increased β-oxidation was found. This means, that the other tissues react on the rapamycin treatment oppositely in long-term, so that the direct effects on skeletal muscle, adipose tissue and hepatocytes are masked. It has been proposed, that the observed hyperlipidemia is rather a result of delayed peripheral clearance. It should be also considered that rapamycin inhibits TORC2 in long-term usage. Further research needs to be done on this matter (Brown et al., 2007; Partha Chakrabarti et al., 2010; Cybulski & Hall, 2009a; Morrisett et al., 2003; Sipula et al., 2006).

9. Conclusion

Nitrogen and lipid metabolism need to be connected to keep the cells alive. Nitrogen intake influences lipid metabolism and lipid accumulation, especially through the TOR pathway. The TOR pathway is a highly conserved pathway among the eukaryotic organisms, widely known to regulate various cellular cues in response to nitrogen and amino acid availability. Among other, the TOR pathway also controls lipid metabolism and therefore lipid accumulation. Despite the conservation of the TOR pathway, its outputs based on the same inputs differ considerably among organisms and even cell types. There is still a lot to be discovered about the crosstalks between nitrogen metabolism and the metabolism of lipids.

References

Adeyo, O., Horn, P. J., Lee, S. K., Binns, D. D., Chandrahas, A., Chapman, K. D., & Goodman, J. M. (2011). The yeast lipin orthologue Pah1p is important for biogenesis of lipid droplets. *Journal of Cell Biology*, *192*(6), 1043–1055. https://doi.org/10.1083/jcb.201010111

Aggarwal, D., Fernandez, M. L., & Soliman, G. A. (2006). Rapamycin, an mTOR inhibitor, disrupts triglyceride metabolism in guinea pigs. *Metabolism: Clinical and Experimental*, *55*(6), 794–802. https://doi.org/10.1016/j.metabol.2006.01.017

Bakan, I., & Laplante, M. (2012). Connecting mTORC1 signaling to SREBP-1 activation. In *Current Opinion in Lipidology* (Vol. 23, Issue 3, pp. 226–234). Curr Opin Lipidol. https://doi.org/10.1097/MOL.0b013e328352dd03

Bell, A., Grunder, L., & Sorisky, A. (2000). Rapamycin Inhibits Human Adipocyte Differentiation in Primary Culture. *Obesity Research*, *8*(3), 249–254. https://doi.org/10.1038/oby.2000.29

Boyle, N. R., Page, M. D., Liu, B., Blaby, I. K., Casero, D., Kropat, J., Cokus, S. J., Hong-Hermesdorf, A., Shaw, J., Karpowicz, S. J., Gallaher, S. D., Johnson, S., Benning, C., Pellegrini, M., Grossman, A., & Merchant, S. S. (2012). Three acyltransferases and nitrogen-responsive regulator are implicated in nitrogen starvation-induced triacylglycerol accumulation in Chlamydomonas. *Journal of Biological Chemistry*, *287*(19), 15811–15825. https://doi.org/10.1074/jbc.M111.334052

Bozza, P. T., & Viola, J. P. B. (2010). Lipid droplets in inflammation and cancer. *Prostaglandins Leukotrienes and Essential Fatty Acids*, *82*(4–6), 243–250. https://doi.org/10.1016/j.plefa.2010.02.005

Bracharz, F., Redai, V., Bach, K., Qoura, F., & Brück, T. (2017). The effects of TORC signal interference on lipogenesis in the oleaginous yeast Trichosporon oleaginosus. *BMC Biotechnology*, *17*(1). https://doi.org/10.1186/s12896-017-0348-3

Broach, J. R. (2012). Nutritional control of growth and development in yeast. In *Genetics* (Vol. 192, Issue 1, pp. 73–105). https://doi.org/10.1534/genetics.111.135731

Brown, N. F., Stefanovic-Racic, M., Sipula, I. J., & Perdomo, G. (2007). The mammalian target of rapamycin regulates lipid metabolism in primary cultures of rat hepatocytes. *Metabolism: Clinical and Experimental*, *56*(11), 1500–1507. https://doi.org/10.1016/j.metabol.2007.06.016

Calvisi, D. F., Wang, C., Ho, C., Ladu, S., Lee, S. A., Mattu, S., Destefanis, G., Delogu, S., Zimmermann, A., Ericsson, J., Brozzetti, S., Staniscia, T., Chen, X., Dombrowski, F., & Evert, M. (2011). Increased lipogenesis, induced by AKT-mTORC1-RPS6 signaling, promotes development of human hepatocellular carcinoma. *Gastroenterology*, *140*(3), 1071-1083.e5. https://doi.org/10.1053/j.gastro.2010.12.006

Caron, A., Richard, D., & Laplante, M. (2015). The Roles of mTOR Complexes in Lipid Metabolism. In *Annual Review of Nutrition* (Vol. 35, Issue 1, pp. 321–348). Annual Reviews Inc.

https://doi.org/10.1146/annurev-nutr-071714-034355

Chakrabarti, P., Kim, J. Y., Singh, M., Shin, Y.-K., Kim, J., Kumbrink, J., Wu, Y., Lee, M.-J., Kirsch, K. H., Fried, S. K., & Kandror, K. V. (2013). Insulin Inhibits Lipolysis in Adipocytes via the Evolutionarily Conserved mTORC1-Egr1-ATGL-Mediated Pathway. *Molecular and Cellular Biology*, *33*(18), 3659–3666. https://doi.org/10.1128/mcb.01584-12

Chakrabarti, Partha, English, T., Shi, J., Smas, C. M., & Kandror, K. V. (2010). Mammalian target of rapamycin complex 1 suppresses lipolysis, stimulates lipogenesis, and promotes fat storage. *Diabetes*, *59*(4), 775–781. https://doi.org/10.2337/db09-1602

Cho, H. J., Park, J., Lee, H. W., Lee, Y. S., & Kim, J. B. (2004). Regulation of adipocyte differentiation and insulin action with rapamycin. *Biochemical and Biophysical Research Communications*, *321*(4), 942–948. https://doi.org/10.1016/j.bbrc.2004.07.050

Csaki, L. S., & Reue, K. (2010). Lipins: Multifunctional lipid metabolism proteins. In *Annual Review of Nutrition* (Vol. 30, pp. 257–272). NIH Public Access. https://doi.org/10.1146/annurev.nutr.012809.104729

Cybulski, N., & Hall, M. N. (2009a). TOR complex 2: a signaling pathway of its own. In *Trends in Biochemical Sciences* (Vol. 34, Issue 12, pp. 620–627). Elsevier Current Trends. https://doi.org/10.1016/j.tibs.2009.09.004

Cybulski, N., & Hall, M. N. (2009b). TOR complex 2: a signaling pathway of its own. In *Trends in Biochemical Sciences* (Vol. 34, Issue 12, pp. 620–627). Elsevier. https://doi.org/10.1016/j.tibs.2009.09.004

Dubots, E., Cottier, S., Péli-Gulli, M. P., Jaquenoud, M., Bontron, S., Schneiter, R., & De Virgilio, C. (2014). TORC1 regulates Pah1 phosphatidate phosphatase activity via the Nem1/Spo7 protein phosphatase complex. *PLoS ONE*, *9*(8). https://doi.org/10.1371/journal.pone.0104194

Düvel, K., Yecies, J. L., Menon, S., Raman, P., Lipovsky, A. I., Souza, A. L., Triantafellow, E., Ma, Q., Gorski, R., Cleaver, S., Vander Heiden, M. G., MacKeigan, J. P., Finan, P. M., Clish, C. B., Murphy, L. O., & Manning, B. D. (2010). Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Molecular Cell*, *39*(2), 171–183. https://doi.org/10.1016/j.molcel.2010.06.022

Dzurendova, S., Zimmermann, B., Tafintseva, V., Kohler, A., Ekeberg, D., & Shapaval, V. (2020). The influence of phosphorus source and the nature of nitrogen substrate on the biomass production and lipid accumulation in oleaginous Mucoromycota fungi. *Applied Microbiology and Biotechnology*, *104*(18), 8065–8076. https://doi.org/10.1007/s00253-020-10821-7

François, J., & Parrou, J. L. (2001). Reserve carbohydrates metabolism in the yeast Saccharomyces cerevisiae . *FEMS Microbiology Reviews*, *25*(1), 125–145. https://doi.org/10.1111/j.1574-6976.2001.tb00574.x

Gaubitz, C., Prouteau, M., Kusmider, B., & Loewith, R. (2016). TORC2 Structure and Function. In *Trends in Biochemical Sciences* (Vol. 41, Issue 6, pp. 532–545). Elsevier Ltd.

https://doi.org/10.1016/j.tibs.2016.04.001

Han, M., Xu, Z. yuan, Du, C., Qian, H., & Zhang, W. G. (2016). Effects of nitrogen on the lipid and carotenoid accumulation of oleaginous yeast Sporidiobolus pararoseus. *Bioprocess and Biosystems Engineering*, *39*(9), 1425–1433. https://doi.org/10.1007/s00449-016-1620-y

Jiang, P. L., Pasaribu, B., & Chen, C. S. (2014). Nitrogen-deprivation elevates lipid levels in Symbiodinium spp. by lipid droplet accumulation: Morphological and compositional analyses. *PLoS ONE*, *9*(1). https://doi.org/10.1371/journal.pone.0087416

Jones, K. T., Greer, E. R., Pearce, D., & Ashrafi, K. (2009). Rictor/torc2 regulates Caenorhabditis elegans fat storage, body size, and development through sgk-1. *PLoS Biology*, *7*(3), 0604–0615. https://doi.org/10.1371/journal.pbio.1000060

Kim, J. E., & Chen, J. (2004). Regulation of peroxisome proliferator-activated receptor-γ activity by mammalian target of rapamycin and amino acids in adipogenesis. *Diabetes*, *53*(11), 2748–2756. https://doi.org/10.2337/diabetes.53.11.2748

Komeili, A., Wedaman, K. P., O'Shea, E. K., & Powers, T. (2000). Mechanism of metabolic control: Target of rapamycin signaling links nitrogen quality to the activity of the Rtg1 and Rtg3 transcription factors. *Journal of Cell Biology*, *151*(4), 863–878. https://doi.org/10.1083/jcb.151.4.863

Laplante, M., Sabatini, D. M., & Author, C. B. (2009). An emerging role of mTOR in lipid biosynthesis NIH Public Access Author Manuscript. *Curr Biol*, *19*(22), 1046–1052. https://doi.org/10.1016/j.cub.2009.09.058

Loewith, R., & Hall, M. N. (2011). Target of rapamycin (TOR) in nutrient signaling and growth control. In *Genetics* (Vol. 189, Issue 4, pp. 1177–1201). Oxford University Press. https://doi.org/10.1534/genetics.111.133363

Lopes, P. C., Fuhrmann, A., Sereno, J., Espinoza, D. O., Pereira, M. J., Eriksson, J. W., Reis, F., & Carvalho, E. (2014). Short and long term in vivo effects of Cyclosporine A and Sirolimus on genes and proteins involved in lipid metabolism in Wistar rats. *Metabolism: Clinical and Experimental*, *63*(5), 702–715. https://doi.org/10.1016/j.metabol.2014.02.004

Madeira, J. B., Masuda, C. A., Maya-Monteiro, C. M., Matos, G. S., Montero-Lomelí, M., & Bozaquel-Morais, B. L. (2015). TORC1 Inhibition Induces Lipid Droplet Replenishment in Yeast. *Molecular and Cellular Biology*, *35*(4), 737–746. https://doi.org/10.1128/mcb.01314-14

Mao, Z., & Zhang, W. (2018). Role of mTOR in glucose and lipid metabolism. In *International Journal of Molecular Sciences* (Vol. 19, Issue 7). MDPI AG. https://doi.org/10.3390/ijms19072043

Maya-Monteiro, C. M., Almeida, P. E., D'Ávila, H., Martins, A. S., Rezende, A. P., Castro-Faria-Neto, H., & Bozza, P. T. (2008). Leptin induces macrophage lipid body formation by a phosphatidylinositol 3-kinase- and mammalian target of rapamycin-dependent mechanism. *Journal of Biological Chemistry*, *283*(4), 2203–2210. https://doi.org/10.1074/jbc.M706706200

Mok, K. W., Mruk, D. D., & Cheng, C. Y. (2013). Regulation of Blood-Testis Barrier (BTB) Dynamics

during Spermatogenesis via the " Yin" and " Yang" Effects of Mammalian Target of Rapamycin Complex 1 (mTORC1) and mTORC2. In *International Review of Cell and Molecular Biology* (Vol. 301, pp. 291–358). Elsevier Inc. https://doi.org/10.1016/B978-0-12-407704-1.00006-3

Morrisett, J. D., Abdel-Fattah, G., & Kahan, B. D. (2003). Sirolimus changes lipid concentrations and lipoprotein metabolism in kidney transplant recipients. *Transplantation Proceedings*, *35*(3 SUPPL.), S143–S150. https://doi.org/10.1016/S0041-1345(03)00233-1

Mousley, C. J., Yuan, P., Gaur, N. A., Trettin, K. D., Nile, A. H., Deminoff, S. J., Dewar, B. J., Wolpert, M., MacDonald, J. M., Herman, P. K., Hinnebusch, A. G., & Bankaitis, V. A. (2012). A sterol-binding protein integrates endosomal lipid metabolism with TOR signaling and nitrogen sensing. *Cell*, *148*(4), 702–715. https://doi.org/10.1016/j.cell.2011.12.026

Muro, E., Ekin Atilla-Gokcumen, G., & Eggert, U. S. (2014). Lipids in cell biology: How can we understand them better? In *Molecular Biology of the Cell* (Vol. 25, Issue 12, pp. 1819–1823). American Society for Cell Biology. https://doi.org/10.1091/mbc.E13-09-0516

Norrmén, C., Figlia, G., Lebrun-Julien, F., Pereira, J. A., Trötzmüller, M., Köfeler, H. C., Rantanen, V., Wessig, C., van Deijk, A. L. F., Smit, A. B., Verheijen, M. H. G., Rüegg, M. A., Hall, M. N., & Suter, U. (2014). mTORC1 controls PNS myelination along the mTORC1-RXRγ-SREBP-lipid biosynthesis axis in Schwann cells. *Cell Reports*, *9*(2), 646–660. https://doi.org/10.1016/j.celrep.2014.09.001

Olzmann, J. A., & Carvalho, P. (2019). Dynamics and functions of lipid droplets. In *Nature Reviews Molecular Cell Biology* (Vol. 20, Issue 3, pp. 137–155). Nature Publishing Group. https://doi.org/10.1038/s41580-018-0085-z

Peng, T., Golub, T. R., & Sabatini, D. M. (2002). The Immunosuppressant Rapamycin Mimics a Starvation-Like Signal Distinct from Amino Acid and Glucose Deprivation. *Molecular and Cellular Biology*, *22*(15), 5575–5584. https://doi.org/10.1128/mcb.22.15.5575-5584.2002

Peterson, T. R., Sengupta, S. S., Harris, T. E., Carmack, A. E., Kang, S. A., Balderas, E., Guertin, D. A., Madden, K. L., Carpenter, A. E., Finck, B. N., & Sabatini, D. M. (2011). MTOR complex 1 regulates lipin 1 localization to control the srebp pathway. *Cell*, *146*(3), 408–420. https://doi.org/10.1016/j.cell.2011.06.034

Porstmann, T., Santos, C. R., Griffiths, B., Cully, M., Wu, M., Leevers, S., Griffiths, J. R., Chung, Y. L., & Schulze, A. (2008). SREBP Activity Is Regulated by mTORC1 and Contributes to Akt-Dependent Cell Growth. *Cell Metabolism*, *8*(3), 224–236. https://doi.org/10.1016/j.cmet.2008.07.007

Rosen, E. D., & MacDougald, O. A. (2006). Adipocyte differentiation from the inside out. *Nature Reviews Molecular Cell Biology*, *7*(12), 885–896. https://doi.org/10.1038/nrm2066

Roustan, V., Bakhtiari, S., Roustan, P. J., & Weckwerth, W. (2017). Quantitative in vivo phosphoproteomics reveals reversible signaling processes during nitrogen starvation and recovery in the biofuel model organism Chlamydomonas reinhardtii. *Biotechnology for Biofuels*, *10*(1).

https://doi.org/10.1186/s13068-017-0949-z

Saponaro, C., Gaggini, M., Carli, F., & Gastaldelli, A. (2015). The subtle balance between lipolysis and lipogenesis: A critical point in metabolic homeostasis. In *Nutrients* (Vol. 7, Issue 11, pp. 9453–9474). MDPI AG. https://doi.org/10.3390/nu7115475

Schmollinger, S., Mühlhaus, T., Boyle, N. R., Blaby, I. K., Casero, D., Mettler, T., Moseley, J. L., Kropat, J., Sommer, F., Strenkert, D., Hemme, D., Pellegrini, M., Grossman, A. R., Stitt, M., Schroda, M., & Merchant, S. S. (2014). Nitrogen-sparing mechanisms in Chlamydomonas affect the transcriptome, the proteome, and photosynthetic metabolism. *Plant Cell*, *26*(4), 1410–1435. https://doi.org/10.1105/tpc.113.122523

Schulz-Raffelt, M., Chochois, V., Auroy, P., Cuiné, S., Billon, E., Dauvillée, D., Li-Beisson, Y., & Peltier, G. (2016). Hyper-accumulation of starch and oil in a Chlamydomonas mutant affected in a plant-specific DYRK kinase. *Biotechnology for Biofuels*, *9*(1). https://doi.org/10.1186/s13068-016-0469-2

Sipula, I. J., Brown, N. F., & Perdomo, G. (2006). Rapamycin-mediated inhibition of mammalian target of rapamycin in skeletal muscle cells reduces glucose utilization and increases fatty acid oxidation. *Metabolism: Clinical and Experimental*, *55*(12), 1637–1644. https://doi.org/10.1016/j.metabol.2006.08.002

Soukas, A. A., Kane, E. A., Carr, C. E., Melo, J. A., & Ruvkun, G. (2009). Rictor/TORC2 regulates fat metabolism, feeding, growth, and life span in Caenorhabditis elegans. *Genes and Development*, *23*(4), 496–511. https://doi.org/10.1101/gad.1775409

Tang, S., Xie, J., Wu, W., Yi, B., Liu, L., & Zhang, H. (2020). High ammonia exposure regulates lipid metabolism in the pig skeletal muscle via mTOR pathway. *Science of the Total Environment*, *740*. https://doi.org/10.1016/j.scitotenv.2020.139917

Tesnière, C., Bessière, C., Pradal, M., Sanchez, I., Blondin, B., & Bigey, F. (2019). Relief from nitrogen starvation entails quick unexpected down-regulation of glycolytic/ lipid metabolism genes in enological Saccharomyces cerevisiae. *PLoS ONE*, *14*(4). https://doi.org/10.1371/journal.pone.0215870

Valledor, L., Furuhashi, T., Recuenco-Muñoz, L., Wienkoop, S., & Weckwerth, W. (2014). System-level network analysis of nitrogen starvation and recovery in Chlamydomonas reinhardtii reveals potential new targets for increased lipid accumulation. *Biotechnology for Biofuels*, *7*(1). https://doi.org/10.1186/s13068-014-0171-1

Walther, T. C., & Farese, R. V. (2012). Lipid droplets and cellular lipid metabolism. *Annual Review of Biochemistry*, *81*(1), 687–714. https://doi.org/10.1146/annurev-biochem-061009-102430

Zach, R., Tvarůžková, J., Schätz, M., Tupa, O., Grallert, B., & Převorovský, M. (2018). Mitotic defects in fission yeast lipid metabolism 'cut' mutants are suppressed by ammonium chloride. *FEMS Yeast Research*, *18*(6). https://doi.org/10.1093/femsyr/foy064

Zhang, W., Du, G., Zhou, J., & Chen, J. (2018). Regulation of Sensing, Transportation, and Catabolism of Nitrogen Sources in Saccharomyces cerevisiae . *Microbiology and Molecular Biology Reviews*, *82*(1). https://doi.org/10.1128/mmbr.00040-17