



HAL
open science

Targeting TOR signaling for enhanced lipid productivity in algae

Laura Prioretti, Frédéric Carriere, Ben Field, Luisana Avilan, Marie-Hélène Montané, Benoît Menand, Brigitte Gontero

► **To cite this version:**

Laura Prioretti, Frédéric Carriere, Ben Field, Luisana Avilan, Marie-Hélène Montané, et al.. Targeting TOR signaling for enhanced lipid productivity in algae. *Biochimie*, 2020, 169, pp.12-17. 10.1016/j.biochi.2019.06.016 . hal-02176524

HAL Id: hal-02176524

<https://amu.hal.science/hal-02176524>

Submitted on 4 Feb 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Targeting TOR signaling for enhanced lipid productivity in algae

2
3 Laura Prioretti¹, Frédéric Carriere¹, Ben Field², Luisana Avilan¹, Marie-Hélène
4 Montané², Benoît Menand^{2*}, Brigitte Gontero^{1*}

5
6 ¹Aix Marseille Univ, CNRS, BIP UMR 7281, 31 Chemin Joseph Aiguier, 13402
7 Marseille Cedex 09, France

8 ²Aix Marseille Univ, CEA, CNRS, UMR 7265 BIAM, 163 Avenue de Luminy, 13288
9 Marseille, France

10 *Corresponding authors: bmeunier@imm.cnrs.fr; benoit.menand@univ-amu.fr

11 12 13 **Abstract**

14
15 Microalgae can be used for many applications, including the development of
16 third generation biofuels. However, the trade-off between biomass production and lipid
17 accumulation and productivity still impairs the viable production of sustainable biofuel
18 from microalgae because neutral lipids accumulate under stress conditions. Recently,
19 in the model marine diatom *Phaeodactylum tricornutum*, it was shown that inhibiting the
20 target of rapamycin (TOR) kinase using specific ATP-competitive inhibitors could
21 overcome this issue. We believe that basic knowledge in this rather new field is
22 required to develop innovative strategies to improve neutral lipid accumulation in
23 oleaginous microalgae. In this minireview, we therefore focused on the current
24 research on the TOR signaling pathway with a focus on its control of lipid homeostasis.
25 Since TOR has been well-studied in animals, we first describe the effectors of TOR in
26 animal lipogenesis, adipogenesis and lipolysis. We then propose a tentative model of
27 TOR regulation of TAG accumulation in algae, a process that has hitherto been
28 overlooked.

29 30 31 **1. Importance of lipids in 3rd generation biofuels from microalgae**

32
33 Lipids, together with carbohydrates, proteins and nucleic acids are essential
34 components of living cells. While glycerophospholipids, sphingolipids, glycolipids and
35 sterol lipids are the main components of cell membranes and can also function as
36 signal transducers, triacylglycerols (TAG) constitute a universal form of transient energy
37 storage in the form of fatty acids esterified with glycerol. They are found for instance in
38 the core of lipid droplets in mammalian adipose tissues, in plant seeds, oleaginous
39 yeasts and microalgae. They are the most abundant neutral lipid species in oils of plant
40 origin. Stored TAGs can fuel the cell in times of nutrient limitation. The pathway leading
41 to neutral lipid synthesis is fairly well conserved among eukaryotes: acetyl-CoA derived
42 from carbon-metabolism is the substrate for *de novo* fatty acid (FA) synthesis, FAs are

43 then conjugated to existing lipophilic alcohols through a series of acylation reactions in
44 which diacylglycerol (DAG) is the last intermediate before TAG [1]. A particularity of
45 plant and algae is that the chloroplast is the major site of *de novo* FA synthesis [2].
46 Cellular TAGs and other neutral storage lipids such as steryl esters are stored in lipid
47 droplets, and their content can vary considerably depending on cell type and external
48 environmental cues. For example, plants store TAGs in seeds in order to support the
49 germination and vegetative growth of the next generation, while microalgae and yeast
50 accumulate TAGs during starvation.

51 In the modern world the increasing energy demand from human activities
52 threatens the availability of finite natural resources and the environment due to the
53 release of CO₂ and other pollutants. Against this background, neutral lipids represent a
54 promising renewable resource for the biofuel industry, as well as for human and animal
55 nutrition and health. Oleaginous microorganisms, including certain microalgae, yeasts
56 and bacteria, can accumulate up to 80% of their biomass in the form of TAGs under
57 appropriate conditions [3, 4]. These organisms therefore represent a major resource for
58 the development of third generation biofuels. Algae are a polyphyletic group of
59 photosynthetic organisms that evolved from multiple endosymbiotic events. The
60 endosymbiosis of a cyanobacterium by a non-photosynthetic eukaryote led to the
61 Archaeplastida group of chloroplast-containing eukaryotes which includes red algae,
62 glaucophytes, green algae and land plants. Further endosymbiosis of green or red
63 algae by other non-photosynthetic eukaryotes led to the evolution of diverse groups of
64 algae including euglenoids and diatoms [5]. Illustrating this diversity, diatoms have
65 retained unique characteristics from different domains of life [6] such as the presence
66 of the urea cycle [7], which is typical of animals, and a sterol biosynthetic pathway that
67 uses intermediates from both plant and fungal sterol biosynthesis [8]. The use of
68 microalgae for lipid stock production has many advantages that have been widely
69 explored and described [9]: a broad range of microalgal strains can be exploited for oil
70 production [4, 10]; they can reach high growth rates (1 to 4 divisions per day for the
71 model green alga, *Chlamydomonas reinhardtii* [11]); and by consuming CO₂ they
72 contribute to reducing greenhouse gas emissions. Moreover, because microalgae can
73 use a variety of other inorganic nutrients they can even be exploited for bio-remediation
74 from wastewaters [3]. Since microalgae are able to grow in controlled environments like
75 that of photobioreactors as well as under extreme conditions (high salinity, and across
76 a broad range of temperature and light intensities), they are not subject to seasonal
77 changes and do not necessarily compete with crops for land and water.

78 Microalgae typically produce large amounts of lipids under stress conditions
79 such as nutrient limitation, and most notably under nitrogen (N) starvation. However,
80 these stresses interrupt cell proliferation and therefore reduce total biomass and limit
81 the overall lipid yield. In the last decade there has been a gold rush for the selection of
82 strains or the creation of engineered microorganisms able to accumulate more lipids. In
83 this regard a path frequently beaten by researchers is to knockout or overexpress
84 genes involved in carbon and lipid metabolism [10]. However, the growth of these

85 strains is often compromised and a better knowledge of the regulation of the balance
86 between growth and lipid accumulation is still required. In this context, a new route is
87 represented by the study of the TOR (Target of Rapamycin) kinase pathway [12], which
88 is a central hub in the regulation of various cell cycle processes including the regulation
89 of nutrient and energy homeostasis, lipid synthesis and is conserved in eukaryotes [13].
90 We will first present evidence for the importance of TOR signaling in the regulation of
91 lipid homeostasis from the wealth of studies in animals, and then we will present how
92 manipulation of TOR signaling can improve TAG productivity in algae. Finally, we will
93 discuss how recent studies in photosynthetic eukaryotes, in parallel with discoveries on
94 TOR signaling in animals, can be used to understand how TOR regulates TAG
95 accumulation in algae and, ultimately, to target TOR signaling for increased lipid
96 productivity.

97 98 99 **2. The principal TOR signaling effectors that control lipid metabolism in** 100 **animals**

101
102 TOR (Target of Rapamycin) is a large (280 kDa) multi-domain kinase belonging
103 to the phosphatidylinositol kinase-related kinase (PIKK) family. TOR is inhibited by
104 rapamycin (Fig. 1) through interaction with the 12 kDa TOR-FRB domain (FKBP12-
105 rapamycin-binding domain). Two canonical TOR containing complexes (TORC),
106 TORC1 and TORC2, exist in both mammals and yeast. Recently, different forms of a
107 TORC3 that differ in structure, function and subcellular localization have been
108 discovered in mammals [14-17]. The TORC1 core, which is composed of the TOR
109 kinase, RAPTOR (regulatory associated protein of TOR) and LST8 (lethal with SEC13
110 protein 8), is found in all eukaryotes [18] and is sensitive to the allosteric inhibitor
111 rapamycin, which binds to the FRB domain of TOR. TORC1 is regulated by nutrients
112 such as glucose, amino acids, as well as by growth factors and is inhibited by stress-
113 related signals such as starvation and hypoxia. Conserved functions of TORC1 include
114 the activation of protein, lipid and nucleotide synthesis, as well as promoting
115 progression of the cell cycle. At the same time, TORC1 also inhibits stress-responsive
116 genes and catabolic processes like autophagy [19, 20]. Upstream signals are funneled
117 towards TORC1 via different repressors and activators that regulate TOR intracellular
118 localization and activity. One of the major upstream repressors of TORC1 is the AMP-
119 activated protein kinase (AMPK), a major sensor of cellular energy status via the
120 AMP/ATP and ADP/ATP ratio in mammals [21]. The orthologues of AMPK in yeast
121 (SNF1, sucrose non fermenting 1) and plants (SnRK1, SNF1-related kinase 1) [22, 23]
122 are not responsive to AMP, indicating that there are distinct strategies for energy
123 sensing in different organisms.

124 As a general rule, active TOR in mammals promotes lipogenesis and
125 adipogenesis and inhibits lipolysis [24]. Lipid synthesis and storage are promoted by

126 the SREBP (sterol regulatory element-binding protein) transcription factor, a master
127 regulator of the transcription of lipo- and sterolgenic genes such as fatty acid synthase,
128 acetyl-CoA carboxylase alpha, stearoyl CoA-desaturase, HMG-CoA reductase, and
129 farnesyl diphosphate synthase [13]. SREBP is retained in the membrane of the
130 endoplasmic reticulum where it is unable to activate transcription. In response to sterol
131 depletion SREBP is cleaved and the SREBP DNA-binding domain is imported into the
132 nucleus where it promotes the transcription of lipogenic enzymes [25]. In mammals,
133 TORC1 regulates the activation of SREBP via two main mechanisms: the
134 phosphorylation of (i) the ribosomal S6 kinase 1 (S6K1) to promote lipogenic processes
135 including the processing of SREBP, and (ii) of lipin-1, a phosphatidic acid phosphatase
136 (PAH) that converts phosphatidic acid (PA) into DAG, an important precursor of
137 membrane and neutral lipids. Upon TORC1 inhibition, lipin-1 becomes
138 dephosphorylated and is imported into the nucleus where it inhibits SREBP to down-
139 regulate lipid synthesis. Interestingly, TORC stability and activity is itself dependent on
140 PA, which binds the FRB domain of TOR and acts as an indicator of nutrient sufficiency
141 [26, 27]. Another S6K1 target, the Serine/Arginine (SR)-rich protein kinase 2 (SRPK2),
142 which controls the expression of lipogenic enzymes was recently reported [28]. It
143 mediates the phosphorylation of SR proteins and their interaction with the spliceosomal
144 protein U1-70K, which is responsible for the splicing of lipogenic pre-mRNAs. The
145 phosphorylation of another mTORC1-S6K target, the glutamylprolyl tRNA synthetase
146 (EPRS), induces EPRS release from the amino acyl tRNA multisynthetase complex
147 and its interaction with the fatty acid transport protein 1, promoting its translocation to
148 the plasma membrane and importing fatty acid to the cells [29]. In addition to inducing
149 lipogenesis and controlling cellular lipid homeostasis in different cell types in the brain
150 and liver, mammalian TORC1 also has a positive effect on the differentiation of stem
151 cells into mature adipocytes and in the formation of adipose tissue [30]. TORC1
152 controls lipid metabolism in white and brown adipose tissues that respectively favor
153 lipid storage or lipid use. The mobilization of the energy stored in the form of TAG,
154 through the action of several lipases [31] and β -oxidation of FA can generate either
155 ATP or heat through the uncoupling protein 1 (UCP1) that decouples the mitochondrial
156 electrochemical gradient. These processes, which are still under study, illustrate the
157 fundamental role of TOR in regulating lipid and energy metabolism at the cell and
158 organism level.

159

160 **3. TOR inhibition can induce a “get fat growth” regime in algae**

161

162 Despite the large diversity and the complex evolution of photosynthetic
163 eukaryotes, all plants and algae investigated contain a conserved TORC1 with at least
164 TOR, RAPTOR and LST8 [32]. No other TOR complexes have yet been described in
165 these groups of organisms. TOR is an essential regulator of proliferation in both plants
166 and algae as shown by the embryo-lethal phenotype of *tor* null mutants in plants, and

167 the repression of both plant and algal growth by the allosteric TOR inhibitor rapamycin
168 or by ATP-competitive TOR inhibitors [33-36].

169 Two independent studies conducted in plants and algae demonstrated the link
170 between TOR repression and neutral lipid accumulation [37, 38]. Repression of *tor*
171 expression by inducible RNA silencing in *Arabidopsis* seedlings led to major metabolic
172 changes including the massive accumulation of storage lipids and starch [37].
173 Likewise, repression of TOR activity by rapamycin treatment led to increased levels in
174 TAG synthesis-related genes, such as glycerol-3-phosphate acyltransferase and acyl-
175 CoA:diacylglycerol acyltransferase (DGAT), and consequently to TAG accumulation in
176 *Cyanidioschyzon merolae* (red algae) and *Chlamydomonas reinhardtii* (green algae)
177 [39]. These studies were pioneering in that they opened the way to targeting TOR
178 signaling for improving plant and algal lipid production. More recent studies conducted
179 on other diverse groups of microalgae, including the diatom *Phaeodactylum tricornutum*
180 and the euglenoid *Euglena gracilis*, showed that TOR inhibition results in the
181 accumulation of large lipid droplets rather similar to those that appear in response to N
182 starvation [34, 39-42] (Fig. 2). TOR inhibition also induces lipid droplet accumulation in
183 budding yeast, suggesting that this phenotype might be a common feature rather than
184 being specific to photosynthetic eukaryotes [40]. Although TAG accumulation seems to
185 be a general response to TOR inhibition in algae and plants, other associated
186 phenotypes are not always similar. For example, while we observed that *P. tricornutum*
187 treatment with the ATP-competitive TOR inhibitor AZD-8055 (Fig. 1) could lead to a
188 stronger decrease in chlorophyll fluorescence than N starvation, no significant effect of
189 rapamycin on chlorophyll content was observed in *C. reinhardtii* by Roustan and
190 Weckwerth [43]. However, while ATP-competitive TOR inhibitors can efficiently inhibit
191 TOR, rapamycin is less efficient, at least in animals where it only partially inhibits TOR
192 function [44]. This is probably also the case in algae since rapamycin, even at high
193 concentrations, only partially represses algal growth to 40-50% of that of untreated
194 cells [34, 35, 45, 46]. On the other hand, ATP-competitive TOR inhibitors can almost
195 completely stop algal proliferation [18, 34, 41]. More comparative analyses with similar
196 TOR inhibitors and at similar effective doses are therefore required to accurately
197 compare the extent and diversity of TOR functions in different algae.

198 As discussed above, a major bottleneck in the development of algae for biofuel
199 production is the fact that most conditions that induce TAG accumulation also repress
200 growth, which strongly limits productivity. Obviously, this is also a concern with TOR
201 inhibitors considering the essential and conserved positive function of TOR in the
202 regulation of cell growth and proliferation. However, analysis of the dose-dependent
203 effects of AZD-8055 in the diatom *P. tricornutum* allowed the identification of a dose
204 that induced a 3 to 4 fold higher lipid productivity than N starvation. This enhanced lipid
205 productivity was due to a moderated impairment of proliferation that allowed cells to
206 grow and divide while accumulating TAG, in what was coined a “get fat-growth regime”
207 [34]. Notably, it is not possible to titrate N to achieve the same effect, because N will be
208 rapidly consumed by the cells. These results show that fine tuning of TOR activity can

209 improve lipid productivity, and underline the need to fully decipher the TOR signaling
210 axis involved in lipid homeostasis to improve the balance between proliferation and
211 TAG accumulation.

212

213

214 **4. How could the TOR signaling pathway regulate neutral lipid accumulation** 215 **in algae?**

216

217 Recent phosphoproteomics and pharmacogenetics analyses have identified
218 potential members of the TOR pathway in photosynthetic eukaryotes. Although some of
219 these data were obtained in *Arabidopsis* and have not yet been confirmed in
220 *Chlamydomonas* or other more distantly related algae, this section presents an
221 overview of the potentially conserved TOR signaling axis involved in lipid homeostasis,
222 and a tentative model of how TOR might regulate TAG accumulation in algae (Fig. 3).

223 S6Ks are conserved TOR targets found in plants and algae that are known to
224 regulate translation [47, 48]. Phosphoproteomic studies of *Chlamydomonas* cells
225 treated with rapamycin or AZD-8055 identified TOR-dependent phosphorylation sites in
226 S6K and ribosomal S6 protein [43, 49]. In mammals, S6K regulates SREBP processing
227 and other lipogenic genes. However, SREBP homologs are not found in plants and
228 algae. Interestingly, one of the two *Arabidopsis* S6Ks localizes to the nucleus,
229 suggesting its potential involvement in transcriptional regulation [50]. Orthologues of
230 the SREBP repressor and PAH (lipin-1) are also found in plants and algae [51].
231 *Arabidopsis* has two PAHs (PAH1 and 2), whose loss of function induces increase in
232 phospholipid content [51]. In algae species, transcriptomics, proteomics or
233 metabolomics did not report PAHs or PA but this might reflect that such signaling
234 proteins and PA are hard to detect in the nutrient conditions devoted to increase
235 cellular lipid contents [52]. In the green microalga *Chlorella vulgaris*, proteomics studies
236 reported that S6K increased under nutrient depleted conditions [53] and the
237 phosphatidic acid phosphatase PAP was identified [54]. Strikingly, PAH2 is
238 phosphorylated in a TOR dependent manner in *Arabidopsis*, suggesting a conservation
239 of the link between TOR and PAH in photosynthetic eukaryotes [55]. PAH1 is also
240 phosphorylated by the cyclin-dependent kinases (CDKs) in *Arabidopsis* suggesting a
241 further link between lipid homeostasis and cell cycle progression [56]. Hence, although
242 a direct link between S6K/PAH and TAG accumulation has not yet been demonstrated
243 in algae, their conservation strongly suggests that they may play a significant role in
244 algal lipid homeostasis (Fig. 3).

245 Other potential effectors of TOR signaling were directly identified in genetic
246 screens on algae. First, two members of the DYRK (dual-specificity tyrosine-
247 phosphorylation regulated kinase) family of protein kinases were isolated in screens for
248 *Chlamydomonas* insertional mutants defective for oil and starch accumulation [57, 58].
249 One, named TAR1 (triacylglycerol accumulation regulator 1) is the homolog of the

250 yeast and *Arabidopsis* YAK1 (yet another kinase 1), and the other, named DYRKP,
251 belongs to a plant and algal DYRK subfamily. A link between YAK1/DYRK and TOR
252 signaling was revealed recently in plants during a screen for *Arabidopsis* mutants
253 resistant to the TOR inhibitor AZD-8055. *Arabidopsis* YAK1 is a negative regulator of
254 growth that is repressed by TOR [59], interacts with TOR, and is phosphorylated in a
255 TOR-dependent manner [55] but it is not yet clear how TOR-YAK/DYRK signaling
256 affects TAG levels in algae. Another screen aimed at identifying TOR signaling
257 effectors in algae led to the identification of the rapamycin-hypersensitive mutant *vip1-1*
258 [60]. VIP1 encodes an inositol hexakisphosphate kinase that pyrophosphorylates the
259 inositol phosphate InsP6 to produce the signaling molecules InsP7 and InsP8. The fact
260 that InsP7 and InsP8 levels drop in rapamycin-treated wild-type cells confirmed a link
261 with TOR signaling [60]. Interestingly, the higher levels of TAGs in the *vip1-1* mutant led
262 to the conclusion that InsP7 and InsP8 activate growth at the expense of TAG
263 accumulation [60]. In *Arabidopsis*, AZD-8055 leads to increased phosphorylation of the
264 VIP1 homolog VIP2 suggesting an indirect regulation by TOR [55]. Interestingly, both
265 *tar1* and *vip1* mutant phenotypes are dependent on the presence of a carbon source.
266 *tar1* mutant fails to accumulate TAGs when N starvation is coupled to acetate supply
267 (photomixotrophy) but accumulates high level of TAGs in photoautotrophic N-deficient
268 conditions [61]. Similarly, *vip1* is hypersensitive to TOR inhibitors on acetate supplied
269 media but not in photoautotrophic conditions [60]. This highlights the importance of the
270 TOR signaling pathway in integrating different nutrient signals to regulate growth and
271 reserve accumulation in algae.

272 Last but not least, autophagy was the first intracellular process connected to
273 TOR signaling to be reported in algae. Rapamycin treatment was shown to induce
274 vacuolization [35] and post-translational modification of the key autophagy-related
275 protein ATG8 in *C. reinhardtii* [62]. More recently, ATG7 phosphorylation was shown to
276 be dependent on TOR [46], suggesting that TOR may regulate autophagy via this
277 protein. In addition, a recent report showed that treatment of cells with concanamycin
278 A, an inhibitor of the autophagic flux, repressed TAG accumulation in response to N
279 starvation. This work indicates that autophagy and vacuolar function are therefore
280 required for lipid droplet accumulation in *Chlamydomonas* [63]. It is therefore likely that
281 the activation of autophagy plays an important role in lipid droplet accumulation in
282 response to TOR inhibition.

283

284

285 **Perspectives**

286

287 This minireview shows the importance of measuring proliferation and/or growth
288 in connection with TAG productivity whatever the context. Manipulation of specific TOR
289 signaling axis and the use of different ATP competitive inhibitors of TOR, at different
290 concentrations, may help to achieve a better TAG productivity. Identifying the

291 downstream targets of TOR needs further investigations to decipher the top secret of
292 TOR in different organisms and especially in algae that are still overlooked for TOR
293 function. More comparative studies are indeed required, particularly between different
294 algae due to their diversity and because most TOR effectors were studied in the model
295 green alga *Chlamydomonas reinhardtii*, which is not necessarily the best choice for
296 biotechnological applications. It is obvious that there are still many remaining questions
297 to understand the relationships between TOR and TAG accumulation in algae and
298 many studies to perform in that fascinating field.

299

300

301 **Acknowledgments**

302
303 This work was supported by the ANR SIGNAUX BioNRJ (ANR-15-CE05-0021-
304 03)

305
306
307 **References**

308
309 [1] K.V. Ruggles, A. Turkish, S.L. Sturley, Making, baking, and breaking: the synthesis, storage, and
310 hydrolysis of neutral lipids, *Annual review of nutrition*, 33 (2013) 413-451.
311 [2] Y. Li-Beisson, J.J. Thelen, E. Fedosejevs, J.L. Harwood, The lipid biochemistry of eukaryotic algae,
312 *Progress in lipid research*, 74 (2019) 31-68.
313 [3] S.A. Shields-Menard, M. Amirsadeghi, W.T. French, R. Boopathy, A review on microbial lipids as a
314 potential biofuel, *Bioresource Technology*, 259 (2018) 451-460.
315 [4] Y. Chisti, Biodiesel from microalgae, *Biotechnology advances*, 25 (2007) 294-306.
316 [5] T. Cavalier-Smith, Principles of protein and lipid targeting in secondary symbiogenesis: euglenoid,
317 dinoflagellate, and sporozoan plastid origins and the eukaryote family tree, *The Journal of eukaryotic*
318 *microbiology*, 46 (1999) 347-366.
319 [6] A.S. Benoiston, F.M. Ibarbalz, L. Bittner, L. Guidi, O. Jahn, S. Dutkiewicz, C. Bowler, The evolution of
320 diatoms and their biogeochemical functions, *Philosophical transactions of the Royal Society of London.*
321 *Series B, Biological sciences*, 372 (2017).
322 [7] A.E. Allen, C.L. Dupont, M. Oborník, A. Horák, A. Nunes-Nesi, J.P. McCrow, H. Zheng, D.A. Johnson, H.
323 Hu, A.R. Fernie, C. Bowler, Evolution and metabolic significance of the urea cycle in photosynthetic
324 diatoms, *Nature*, 473 (2011) 203.
325 [8] M. Fabris, M. Matthijs, S. Carbonelle, T. Moses, J. Pollier, R. Dasseville, G.J.E. Baart, W. Vyverman, A.
326 Goossens, Tracking the sterol biosynthesis pathway of the diatom *Phaeodactylum tricornutum*, *New*
327 *Phytologist*, 204 (2014) 521-535.
328 [9] Q. Hu, M. Sommerfeld, E. Jarvis, M. Ghirardi, M. Posewitz, M. Seibert, A. Darzins, Microalgal
329 triacylglycerols as feedstocks for biofuel production: perspectives and advances, *The Plant journal : for*
330 *cell and molecular biology*, 54 (2008) 621-639.
331 [10] M.-H. Liang, J.-G. Jiang, Advancing oleaginous microorganisms to produce lipid via metabolic
332 engineering technology, *Progress in lipid research*, 52 (2013) 395-408.
333 [11] Z.T. Wang, N. Ullrich, S. Joo, S. Waffenschmidt, U. Goodenough, Algal lipid bodies: stress induction,
334 purification, and biochemical characterization in wild-type and starchless *Chlamydomonas reinhardtii*,
335 *Eukaryotic cell*, 8 (2009) 1856-1868.
336 [12] R. Loewith, M.N. Hall, Target of rapamycin (TOR) in nutrient signaling and growth control, *Genetics*,
337 189 (2011) 1177-1201.
338 [13] A. Caron, D. Richard, M. Laplante, The Roles of mTOR Complexes in Lipid Metabolism, *Annual*
339 *review of nutrition*, 35 (2015) 321-348.
340 [14] F.C. Harwood, R.I. Klein Geltink, B.P. O'Hara, M. Cardone, L. Janke, D. Finkelstein, I. Entin, L. Paul,
341 P.J. Houghton, G.C. Grosveld, ETV7 is an essential component of a rapamycin-insensitive mTOR complex
342 in cancer, *Science advances*, 4 (2018) eaar3938.
343 [15] D. Kazyken, Y. Kaz, V. Kiyani, A.A. Zhylykbayev, C.H. Chen, N.K. Agarwal, D. Sarbassov dos, The
344 nuclear import of ribosomal proteins is regulated by mTOR, *Oncotarget*, 5 (2014) 9577-9593.
345 [16] Y. Luo, L. Liu, Y. Wu, K. Singh, B. Su, N. Zhang, X. Liu, Y. Shen, S. Huang, Rapamycin inhibits mSin1
346 phosphorylation independently of mTORC1 and mTORC2, *Oncotarget*, 6 (2015) 4286-4298.
347 [17] L.J. Smithson, D.H. Gutmann, Proteomic analysis reveals GIT1 as a novel mTOR complex component
348 critical for mediating astrocyte survival, *Genes & development*, 30 (2016) 1383-1388.

349 [18] M.H. Montane, B. Menand, TOR inhibitors: from mammalian outcomes to pharmacogenetics in
350 plants and algae, *Journal of experimental botany*, (2019).

351 [19] M. Laplante, D.M. Sabatini, mTOR signaling in growth control and disease, *Cell*, 149 (2012) 274-
352 293.

353 [20] R.A. Saxton, D.M. Sabatini, mTOR Signaling in Growth, Metabolism, and Disease, *Cell*, 168 (2017)
354 960-976.

355 [21] P. Crozet, L. Margalha, A. Confraria, A. Rodrigues, C. Martinho, M. Adamo, C.A. Elias, E. Baena-
356 Gonzalez, Mechanisms of regulation of SNF1/AMPK/SnRK1 protein kinases, *Frontiers in plant science*, 5
357 (2014) 190.

358 [22] K.M. Jamsheer, B.N. Shukla, S. Jindal, N. Gopan, C.T. Mannully, A. Laxmi, The FCS-like zinc finger
359 scaffold of the kinase SnRK1 is formed by the coordinated actions of the FLZ domain and intrinsically
360 disordered regions, *The Journal of biological chemistry*, 293 (2018) 13134-13150.

361 [23] P. Sanz, R. Viana, M.A. Garcia-Gimeno, AMPK in Yeast: The SNF1 (Sucrose Non-fermenting 1)
362 Protein Kinase Complex, *Experientia supplementum* (2012), 107 (2016) 353-374.

363 [24] S.J. Ricoult, B.D. Manning, The multifaceted role of mTORC1 in the control of lipid metabolism,
364 *EMBO reports*, 14 (2013) 242-251.

365 [25] T.I. Jeon, T.F. Osborne, SREBPs: metabolic integrators in physiology and metabolism, *Trends in*
366 *endocrinology and metabolism: TEM*, 23 (2012) 65-72.

367 [26] D. Menon, D. Salloum, E. Bernfeld, E. Gorodetsky, A. Akselrod, M.A. Frias, J. Sudderth, P.H. Chen, R.
368 DeBerardinis, D.A. Foster, Lipid sensing by mTOR complexes via de novo synthesis of phosphatidic acid,
369 *The Journal of biological chemistry*, 292 (2017) 6303-6311.

370 [27] M.S. Yoon, Y. Sun, E. Arauz, Y. Jiang, J. Chen, Phosphatidic acid activates mammalian target of
371 rapamycin complex 1 (mTORC1) kinase by displacing FK506 binding protein 38 (FKBP38) and exerting an
372 allosteric effect, *The Journal of biological chemistry*, 286 (2011) 29568-29574.

373 [28] G. Lee, Y. Zheng, S. Cho, C. Jang, C. England, J.M. Dempsey, Y. Yu, X. Liu, L. He, P.M. Cavaliere, A.
374 Chavez, E. Zhang, M. Isik, A. Couvillon, N.E. Dephoure, T.K. Blackwell, J.J. Yu, J.D. Rabinowitz, L.C.
375 Cantley, J. Blenis, Post-transcriptional Regulation of De Novo Lipogenesis by mTORC1-S6K1-SRPK2
376 Signaling, *Cell*, 171 (2017) 1545-1558 e1518.

377 [29] A. Arif, F. Terenzi, A.A. Potdar, J. Jia, J. Sacks, A. China, D. Halawani, K. Vasu, X. Li, J.M. Brown, J.
378 Chen, S.C. Kozma, G. Thomas, P.L. Fox, EPRS is a critical mTORC1-S6K1 effector that influences adiposity
379 in mice, *Nature*, 542 (2017) 357-361.

380 [30] M.F. Wiperman, D.C. Montrose, A.M. Gotto, Jr., D.P. Hajjar, Mammalian Target of Rapamycin: A
381 Metabolic Rheostat for Regulating Adipose Tissue Function and Cardiovascular Health, *The American*
382 *journal of pathology*, 189 (2019) 492-501.

383 [31] S. D'Andrea, Lipid droplet mobilization: The different ways to loosen the purse strings, *Biochimie*,
384 120 (2016) 17-27.

385 [32] T.J. van Dam, F.J. Zwartkruis, J.L. Bos, B. Snel, Evolution of the TOR pathway, *Journal of molecular*
386 *evolution*, 73 (2011) 209-220.

387 [33] M.H. Montané, B. Menand, ATP-competitive mTOR kinase inhibitors delay plant growth by
388 triggering early differentiation of meristematic cells but no developmental patterning change, *Journal*
389 *of experimental botany*, 64 (2013) 4361-4374.

390 [34] L. Prioretti, L. Avilan, F. Carriere, M.-H. Montane, B. Field, G. GREGORI, B. Menand, B. Gontero, The
391 inhibition of TOR in the model diatom *Phaeodactylum tricornutum* promotes a get-fat growth regime,
392 *Algal Research - Biomass, Biofuels and Bioproducts*, 26 (2017) 265-274.

393 [35] J.L. Crespo, S. Diaz-Troya, F.J. Florencio, Inhibition of target of rapamycin signaling by rapamycin in
394 the unicellular green alga *Chlamydomonas reinhardtii*, *Plant Physiol*, 139 (2005) 1736-1749.

395 [36] B. Menand, T. Desnos, L. Nussaume, F. Berger, D. Bouchez, C. Meyer, C. Robaglia, Expression and
396 disruption of the Arabidopsis TOR (target of rapamycin) gene, *Proceedings of the National Academy of*
397 *Sciences of the United States of America*, 99 (2002) 6422-6427.

398 [37] C. Caldana, Y. Li, A. Leisse, Y. Zhang, L. Bartholomaeus, A.R. Fernie, L. Willmitzer, P. Giavalisco,
399 Systemic analysis of inducible target of rapamycin mutants reveal a general metabolic switch controlling
400 growth in *Arabidopsis thaliana*, *The Plant journal : for cell and molecular biology*, 73 (2013) 897-909.

401 [38] D.Y. Lee, O. Fiehn, Metabolomic response of *Chlamydomonas reinhardtii* to the inhibition of target
402 of rapamycin (TOR) by rapamycin, *Journal of microbiology and biotechnology*, 23 (2013) 923-931.

403 [39] S. Imamura, Y. Kawase, I. Kobayashi, T. Sone, A. Era, S.Y. Miyagishima, M. Shimojima, H. Ohta, K.
404 Tanaka, Target of rapamycin (TOR) plays a critical role in triacylglycerol accumulation in microalgae,
405 *Plant molecular biology*, 89 (2015) 309-318.

406 [40] J.B. Madeira, C.A. Masuda, C.M. Maya-Monteiro, G.S. Matos, M. Montero-Lomeli, B.L. Bozaquel-
407 Morais, TORC1 inhibition induces lipid droplet replenishment in yeast, *Molecular and cellular biology*,
408 35 (2015) 737-746.

409 [41] S. Imamura, Y. Kawase, I. Kobayashi, M. Shimojima, H. Ohta, K. Tanaka, TOR (target of rapamycin) is
410 a key regulator of triacylglycerol accumulation in microalgae, *Plant signaling & behavior*, 11 (2016)
411 e1149285.

412 [42] S. Mukaida, T. Ogawa, K. Ohishi, Y. Tanizawa, D. Ohta, M. Arita, The effect of rapamycin on
413 biodiesel-producing protist *Euglena gracilis*, *Bioscience, biotechnology, and biochemistry*, 80 (2016)
414 1223-1229.

415 [43] V. Roustan, W. Weckwerth, Quantitative Phosphoproteomic and System-Level Analysis of TOR
416 Inhibition Unravel Distinct Organellar Acclimation in *Chlamydomonas reinhardtii*, *Frontiers in plant
417 science*, 9 (2018) 1590.

418 [44] S.A. Kang, M.E. Pacold, C.L. Cervantes, D. Lim, H.J. Lou, K. Ottina, N.S. Gray, B.E. Turk, M.B. Yaffe,
419 D.M. Sabatini, mTORC1 phosphorylation sites encode their sensitivity to starvation and rapamycin,
420 *Science (New York, N.Y.)*, 341 (2013) 1236566.

421 [45] J. Juppner, U. Mubeen, A. Leisse, C. Caldana, A. Wiszniewski, D. Steinhäuser, P. Giavalisco, The
422 target of rapamycin kinase affects biomass accumulation and cell cycle progression by altering
423 carbon/nitrogen balance in synchronized *Chlamydomonas reinhardtii* cells, *The Plant journal : for cell
424 and molecular biology*, 93 (2018) 355-376.

425 [46] V. Roustan, S. Bakhtiari, P.-J. Roustan, W. Weckwerth, Quantitative in vivo phosphoproteomics
426 reveals reversible signaling processes during nitrogen starvation and recovery in the biofuel model
427 organism *Chlamydomonas reinhardtii*, *Biotechnology for Biofuels*, 10 (2017) 280.

428 [47] T. Dobrenel, E. Mancera-Martínez, C. Forzani, M. Azzopardi, M. Davanture, M. Moreau, M.
429 Schepetilnikov, J. Chicher, O. Langella, M. Zivy, C. Robaglia, L.A. Ryabova, J. Hanson, C. Meyer, The
430 *Arabidopsis* TOR Kinase Specifically Regulates the Expression of Nuclear Genes Coding for Plastidic
431 Ribosomal Proteins and the Phosphorylation of the Cytosolic Ribosomal Protein S6, *Frontiers in plant
432 science*, 7 (2016) 1611.

433 [48] J. Serfontein, R.E. Nisbet, C.J. Howe, P.J. de Vries, Evolution of the TSC1/TSC2-TOR signaling
434 pathway, *Science signaling*, 3 (2010) ra49.

435 [49] E.G. Werth, E.W. McConnell, I. Couso Lianez, Z. Perrine, J.L. Crespo, J.G. Umen, L.M. Hicks,
436 Investigating the effect of target of rapamycin kinase inhibition on the *Chlamydomonas reinhardtii*
437 phosphoproteome: from known homologs to new targets, *New Phytologist*, 221 (2019) 247-260.

438 [50] M.M. Mahfouz, S. Kim, A.J. Delauney, D.P. Verma, *Arabidopsis* TARGET OF RAPAMYCIN interacts
439 with RAPTOR, which regulates the activity of S6 kinase in response to osmotic stress signals, *The Plant
440 cell*, 18 (2006) 477-490.

441 [51] Y. Nakamura, R. Koizumi, G. Shui, M. Shimojima, M.R. Wenk, T. Ito, H. Ohta, *Arabidopsis* lipins
442 mediate eukaryotic pathway of lipid metabolism and cope critically with phosphate starvation,
443 *Proceedings of the National Academy of Sciences of the United States of America*, 106 (2009) 20978-
444 20983.

445 [52] N. Arora, P.T. Pienkos, V. Pruthi, K.M. Poluri, M.T. Guarnieri, Leveraging algal omics to reveal
446 potential targets for augmenting TAG accumulation, *Biotechnology advances*, 36 (2018) 1274-1292.

- 447 [53] M.T. Guarnieri, A. Nag, S. Yang, P.T. Pienkos, Proteomic analysis of *Chlorella vulgaris*: potential
448 targets for enhanced lipid accumulation, *Journal of proteomics*, 93 (2013) 245-253.
- 449 [54] M.T. Guarnieri, A. Nag, S.L. Smolinski, A. Darzins, M. Seibert, P.T. Pienkos, Examination of
450 triacylglycerol biosynthetic pathways via de novo transcriptomic and proteomic analyses in an
451 unsequenced microalga, *PloS one*, 6 (2011) e25851.
- 452 [55] J. Van Leene, C. Han, A. Gadeyne, D. Eeckhout, C. Matthijs, B. Cannoot, N. De Winne, G. Persiau, E.
453 Van De Slijke, B. Van de Cotte, E. Stes, M. Van Bel, V. Storme, F. Impens, K. Gevaert, K. Vandepoele, I.
454 De Smet, G. De Jaeger, Capturing the phosphorylation and protein interaction landscape of the plant
455 TOR kinase, *Nature plants*, 5 (2019) 316-327.
- 456 [56] C.P. Craddock, N. Adams, J.T. Kroon, F.M. Bryant, P.J. Hussey, S. Kurup, P.J. Eastmond, Cyclin-
457 dependent kinase activity enhances phosphatidylcholine biosynthesis in *Arabidopsis* by repressing
458 phosphatidic acid phosphohydrolase activity, *The Plant journal : for cell and molecular biology*, 89
459 (2017) 3-14.
- 460 [57] M. Kajikawa, Y. Sawaragi, H. Shinkawa, T. Yamano, A. Ando, M. Kato, M. Hirono, N. Sato, H.
461 Fukuzawa, Algal dual-specificity tyrosine phosphorylation-regulated kinase, triacylglycerol accumulation
462 regulator1, regulates accumulation of triacylglycerol in nitrogen or sulfur deficiency, *Plant Physiol*, 168
463 (2015) 752-764.
- 464 [58] M. Schulz-Raffelt, V. Chochois, P. Auroy, S. Cuine, E. Billon, D. Dauvillee, Y. Li-Beisson, G. Peltier,
465 Hyper-accumulation of starch and oil in a *Chlamydomonas* mutant affected in a plant-specific DYRK
466 kinase, *Biotechnol Biofuels*, 9 (2016) 55.
- 467 [59] A. Barrada, M. Djendli, T. Desnos, R. Mercier, C. Robaglia, M.H. Montane, B. Menand, A TOR-YAK1
468 signaling axis controls cell cycle, meristem activity and plant growth in *Arabidopsis*, *Development*, 146
469 (2019).
- 470 [60] I. Couso, B.S. Evans, J. Li, Y. Liu, F. Ma, S. Diamond, D.K. Allen, J.G. Umen, Synergism between
471 Inositol Polyphosphates and TOR Kinase Signaling in Nutrient Sensing, Growth Control, and Lipid
472 Metabolism in *Chlamydomonas*, *The Plant cell*, 28 (2016) 2026-2042.
- 473 [61] H. Shinkawa, M. Kajikawa, Y. Nomura, M. Ogura, Y. Sawaragi, T. Yamano, H. Nakagami, N.
474 Sugiyama, Y. Ishihama, Y. Kanesaki, H. Yoshikawa, H. Fukuzawa, Algal Protein Kinase, Triacylglycerol
475 Accumulation Regulator1, Modulates Cell Viability and Gametogenesis in Carbon/Nitrogen Imbalanced
476 Conditions, *Plant & cell physiology*, (2019).
- 477 [62] M.E. Perez-Perez, I. Couso, J.L. Crespo, The TOR Signaling Network in the Model Unicellular Green
478 Alga *Chlamydomonas reinhardtii*, *Biomolecules*, 7 (2017).
- 479 [63] I. Couso, M.E. Perez-Perez, E. Martinez-Force, H.S. Kim, Y. He, J.G. Umen, J.L. Crespo, Autophagic
480 flux is required for the synthesis of triacylglycerols and ribosomal protein turnover in *Chlamydomonas*,
481 *Journal of experimental botany*, 69 (2018) 1355-1367.

482

483

484 **Figure legends**

485

486 **Fig. 1.** Chemical structures of the TOR inhibitors rapamycin (A) and AZD-8055 (B).

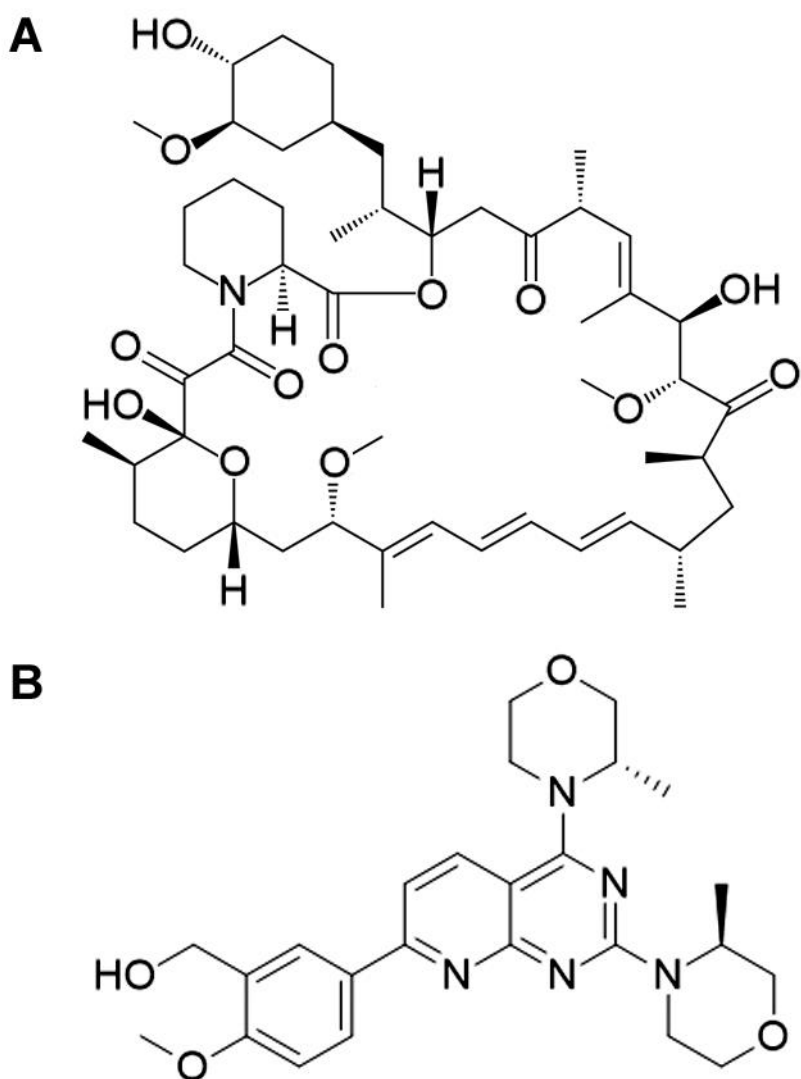
487

488 **Fig. 2.** Effect of TOR inhibition on lipid storage in microalgae. A) Nile Red staining of
489 lipid droplets in *Phaeodactylum tricornutum* after five days of treatment with DMSO
490 (left) or AZD-8055 2 μ M (right), scale bars 10 μ m (modified with permission from
491 Prioretti et al. 2017). B) BODIPY and AC-202 merged staining of lipid droplets in
492 *Chlamydomonas reinhardtii* after 48h of treatment with DMSO (left) or AZD-8055 2 μ M
493 (right), scale bar 5 μ m (modified from Harchouni et al. 2018). C) BODIPY staining of
494 lipid droplets in a rapamycin-sensitive strain of *Cyanidioschyzon merolae* expressing

495 yeast FKBP12 after 48h of treatment with DMSO (left) or rapamycin 1 μ M (right), scale
496 bars 10 μ m (modified with permission from Imamura et al. 2015). D) Nile Red staining
497 of lipid droplets in *Euglena gracilis* after five days of treatment with ethanol (left) or
498 rapamycin 1 μ M (right), scale bars 20 μ m (modified with permission from Mukaida et al.
499 2016).

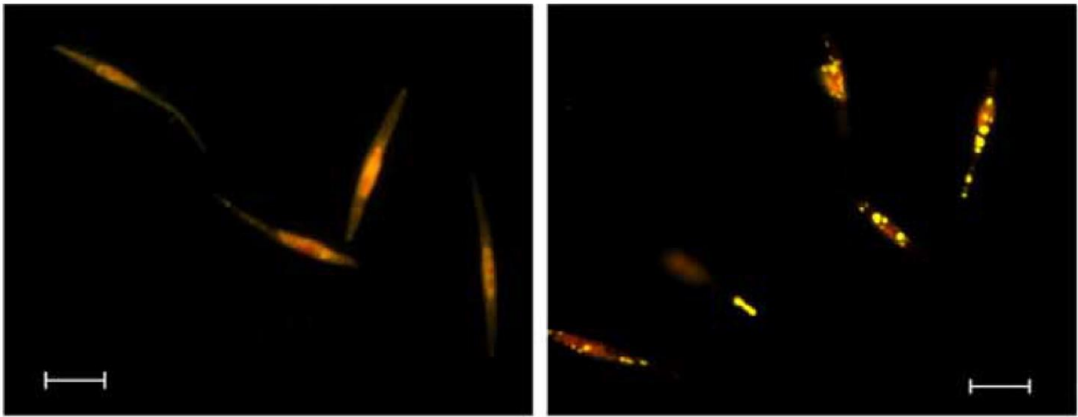
500
501 **Fig. 3.** Model of effectors of TOR-dependent regulation of growth and lipid homeostasis
502 in microalgae.

503
504
505

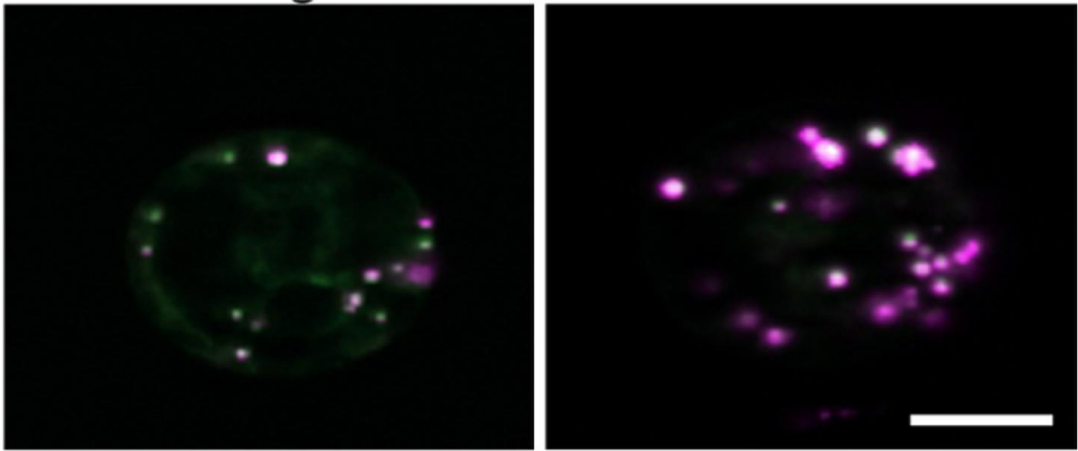


506

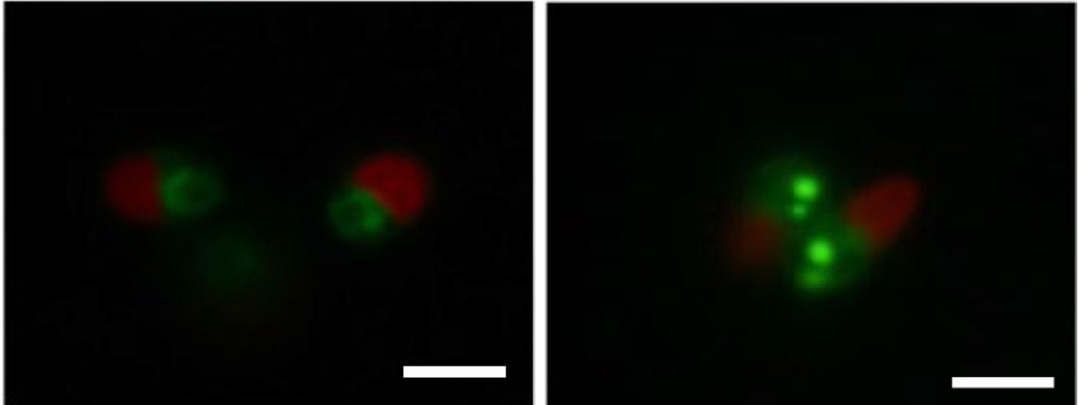
A



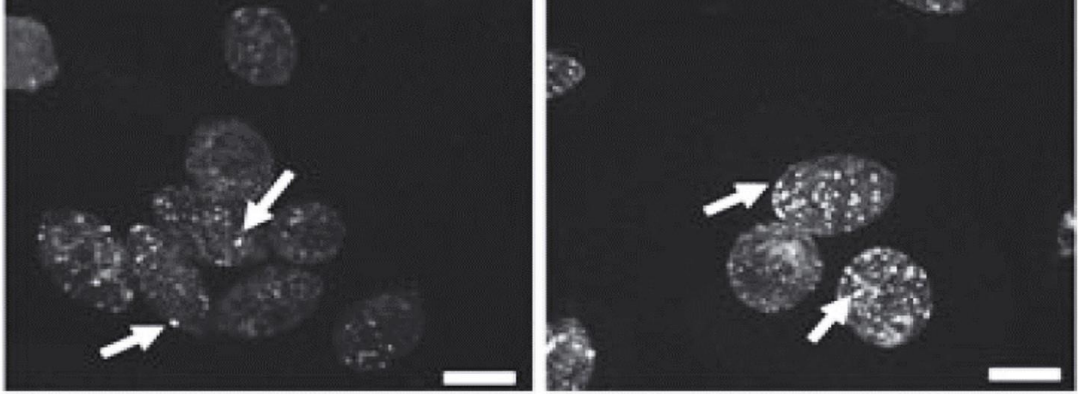
B

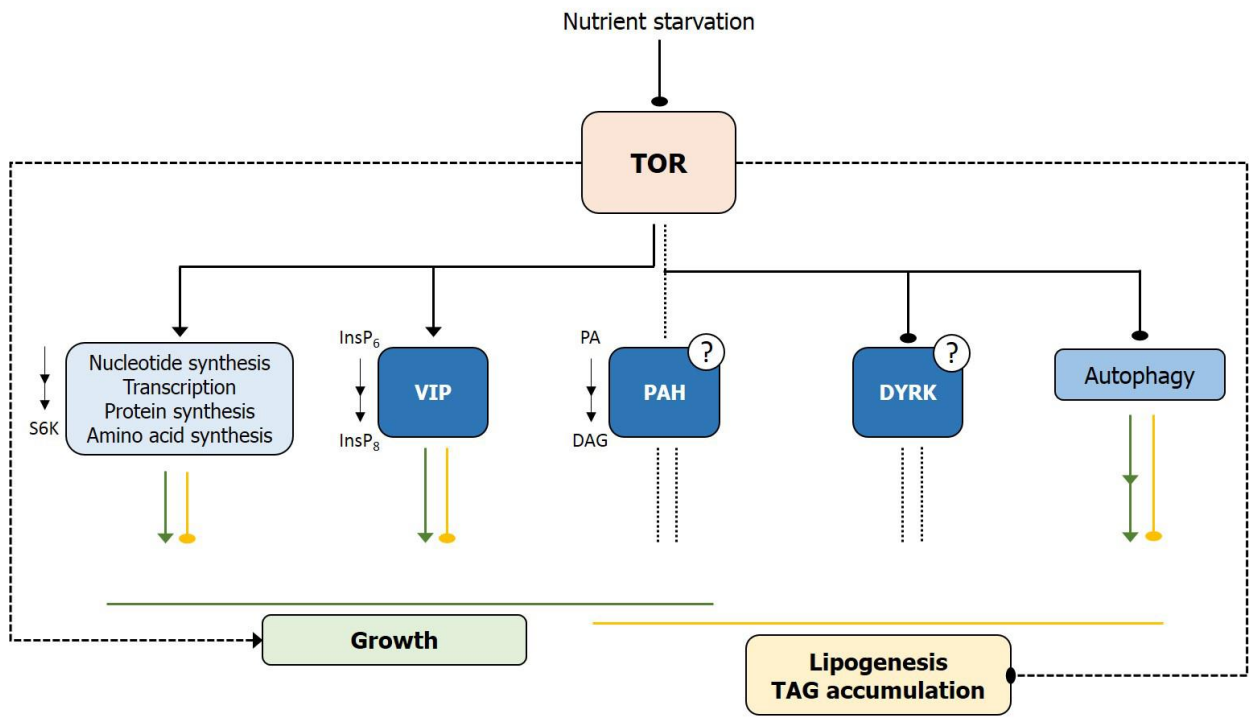


C



D





508