



**HAL**  
open science

## TOR inhibitors: from mammalian outcomes to pharmacogenetics in plants and algae

Marie-Hélène Montané, Benoît Menand

### ► To cite this version:

Marie-Hélène Montané, Benoît Menand. TOR inhibitors: from mammalian outcomes to pharmacogenetics in plants and algae. *Journal of Experimental Botany*, 2019, 70 (8), pp.2297-2312. 10.1093/jxb/erz053 . cea-02073630

**HAL Id: cea-02073630**

**<https://cea.hal.science/cea-02073630>**

Submitted on 17 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 **TOR inhibitors: from mammalian outcomes to pharmacogenetics in plants and**  
2 **algae**

3 Review paper

4

5 Marie-Hélène Montané<sup>1\*</sup> and Benoît Menand<sup>1\*</sup>

6 <sup>1</sup>Aix Marseille Univ, CEA, CNRS, BIAM, Laboratoire de génétique et biophysique des plantes,  
7 Marseille, France F-13009

8 \*correspondance: marie-helene.montane@univ-amu.fr and benoit.menand@univ-amu.fr

9 **Running title:** TOR inhibitors in plant and algae

10 **Keywords:** TOR, mammals, plants, algae, rapamycin, ATP-competitive TOR inhibitor.

11

12 **Abstract**

13 Target Of Rapamycin (TOR) is a conserved eukaryotic phosphatidylinositol 3-kinase (PI3K)-related kinase  
14 (PIKK) that regulates growth and metabolism in response to environment in plants and algae. The study of  
15 the plant and algal TOR pathway largely depends on TOR inhibitors first developed for non-photosynthetic  
16 eukaryotes. In animals and yeast, fundamental works on the TOR pathway have benefited from the  
17 allosteric TOR inhibitor rapamycin and more recently from ATP-competitive TOR inhibitors (asTORis)  
18 that circumvent the limitations of rapamycin. The asTORis, developed for medical applications, inhibit  
19 TORC1 more efficiently than rapamycin and also inhibit rapamycin-resistant TOR complexes (TORCs).  
20 This review will present knowledge on TOR inhibitors from the mammalian field and underline  
21 important consideration for plant and algal biologists. We will discuss the use of rapamycin and

22 asTORis in plants and algae and conclude with guidelines for physiological studies and genetic screens  
23 with TOR inhibitors.

24

## 25 **Introduction**

26 Rapamycin together with the structurally related drug FK506 are immunosuppressive agents that are  
27 reciprocal antagonists of lymphocyte cell activation (Sigal and Dumont, 1992). Rapamycin stood out  
28 for its role in second phase of lymphocyte activation by inhibiting cell cycle and subsequently  
29 proliferation (Aagaard-Tillery and Jelinek, 1994). A recent overview of rapamycin (Yoo *et al.*, 2017)  
30 describes the respective mechanisms of immunosuppressive action of FK506 that interferes with the  
31 phosphatase calcineurin and of rapamycin that interferes with the serine/threonine kinase “Target Of  
32 Rapamycin” (TOR). Both compounds bind to a single domain of the cytosolic immunophilin FKBP12  
33 (12 kDa FK506 Binding Protein). Briefly, the FKBP12-rapamycin duo binds to the so-called FRB  
34 (FKBP12-Rapamycin Binding) domain of TOR therefore creating a ternary complex that inhibits TOR  
35 kinase activity through allosteric interaction. Throughout eukaryotes, TOR progressively emerged as a  
36 hub for orchestrating cellular anabolic and catabolic processes that basically characterize growth  
37 homeostasis, *i.e.*, cell/organ size and cell proliferation as well as cell components turnover. “In simple  
38 terms, cell growth is the accumulation of mass. But this description short changes a process that is  
39 vastly more complex and interesting” (Thoreen, 2017). TOR interconnects numerous inputs and  
40 outputs of anabolism functions while repressing autophagy, ensuring growth homeostasis, *i.e.*, the  
41 building up, the “stability” or survival of cells up to their aging and senescence or in response to any  
42 imbalance caused by stress, disease or energy changes (Saxton and Sabatini, 2017; Thoreen, 2017).  
43 Rapamycin was decisive for the discovery of TOR protein, basic TOR complexes (TORCs)  
44 components and targets (Alessi *et al.*, 2009; Huang *et al.*, 2003), yet the recent development of ATP-  
45 competitive TOR inhibitors (active site TOR inhibitors, asTORis) brought new tools to study more in

46 depth the TOR pathway. Furthermore, since the treatment of cancer by rapamycin and its derivatives  
47 rapalogs gave disappointing results, these second generation inhibitors also provided new possibilities  
48 of clinical trials aiming to cure cancer and other pathologies (Martelli *et al.*, 2018). In the context of  
49 studying TOR functions in plants, we aim to state here the use of rapamycin and of asTORis with an  
50 emphasis on their potential for pharmacogenetic studies in plant and algae.

51

## 52 **Rapamycin and TOR complexes from yeast and mammals to plants and algae**

53 For the historical steps on the discovery and naming of TOR, we invite the reader to rely on very  
54 informative articles of DA Sabatini and MN Hall (Hall, 2016; Sabatini, 2017). The TOR protein kinase  
55 was first identified from a genetic screen of *Saccharomyces cerevisiae* (referred hereafter as yeast)  
56 lines that were resistant to rapamycin (Heitman *et al.*, 1991). Rapamycin-resistant lines mostly carried  
57 recessive missense mutations resulting in amino acid substitutions in the FKBP12 protein, but  
58 dominant missense mutations in two genes named *TOR1* and *TOR2* (Target Of Rapamycin 1 and 2)  
59 were also identified. Further studies revealed that mutations of a conserved Serine residue within the  
60 FRB domain of *TOR1* or *TOR2* confers dominant resistance to rapamycin (Stan *et al.*, 1994). Soon  
61 after, three groups identified the “physical target of rapamycin” in mammals by biochemical  
62 approaches using rapamycin and FKBP12. TOR is a member of the atypical Ser/Thr-protein kinase of  
63 the PIKKs family that all play vital role in growth and survival and also includes essential regulators of  
64 the DNA damage response such as ATM (Ataxia-Telangiectasia Mutated), ATR (ATM- and Rad3-  
65 Related) and DNA-PK (DNA-dependent Protein Kinase) (De Cicco *et al.*, 2015). Rapamycin has been  
66 an indispensable tool for studying the roles of the TOR pathway in both yeast and animals but  
67 rapamycin effects are more limited in animals on protein synthesis, autophagy and proliferation (**Fig.**  
68 **1A**) and varied widely among cell types (Mukhopadhyay *et al.*, 2016; Sarbassov *et al.*, 2006; Thoreen,  
69 2017; Zhao *et al.*, 2015). Both genetic and biochemical studies identified two basic TOR complexes:  
70 The rapamycin sensitive TORC1 containing RAPTOR/KOG1 (mammalian Regulatory Associated

71 Protein of TOR/yeast Kontroller Of Growth 1) and LST8 (Lethal with SEC13 protein 8), and the  
72 rapamycin-insensitive TORC2 containing LST8 and RICTOR/AVO3 (mammalian Rapamycin-  
73 Insensitive Companion of mTOR/yeast Adheres-VOraciously-to-tor-2 protein 3). TORC2 components  
74 and downstream effectors have been difficult to characterize due to the absence of specific drugs that  
75 selectively inhibit this complex (Gaubitz *et al.*, 2016; Sparks and Guertin, 2010) and because under  
76 prolonged (chronic and not acute) rapamycin treatment, TORC2 assembly was impaired (Sarbasov *et*  
77 *al.*, 2006). TORC2 is involved in cell survival and cytoskeleton regulation through different AGC  
78 family kinases including a key readout target kinase AKT, which phosphorylation requires  
79 SIN1/AVO1 (mammalian Stress-activated protein kinase-INteracting protein 1/yeast Adheres-  
80 VOraciously-to-target-of-rapamycin-2 protein 1), another essential component of TORC2 (Gaubitz *et*  
81 *al.*, 2016). Noticeably, SIN1 isoforms led to suggest occurrence of 3 different TORC2, showing  
82 plasticity of TOR complexes. A detailed composition of yeast and mammals TORC1 and TORC2 and  
83 the full range of downstream targets through which TOR drives cell growth has recently fully emerged  
84 and is extensively reviewed elsewhere (Ben-Sahra and Manning, 2017; Eltschinger and Loewith,  
85 2016; Gaubitz *et al.*, 2016; Gonzalez and Rallis, 2017; Jhanwar-Uniyal *et al.*, 2017; Saxton and  
86 Sabatini, 2017).

87         The control of cell growth by TORC1 in response to nutrients was early demonstrated in yeast  
88 (Barbet *et al.*, 1996) and later on transcriptional profiling showed that mammalian TORC1 up-  
89 regulates sets of genes involved in lipid/sterol, nucleotide and protein synthesis, as well as genes  
90 involved in mitochondrial oxidative function, glycolysis and the pentose phosphate pathway and  
91 conversely down-regulated genes involved in starvation and energy production (Duvel *et al.*, 2010;  
92 Peng *et al.*, 2002). Briefly, under adequate conditions including growth factors, amino acids and AMP  
93 to ATP and/or ADP to ATP ratios, TORC1 phosphorylates two foremost targets involved in protein  
94 synthesis commitment and elongation, the eIF4E-Binding Protein1 (4E-BP1) and the ribosomal  
95 protein S6 Kinases (S6Ks) respectively. However, it is worth mentioning that the clear cut contribution

96 of each TORC1-S6K1/S6K2 and TORC1-4E-BP1 axis in regulating cell cycle and proliferation as  
97 well as translation has been hard to delineate (Cunningham *et al.*, 2007; Dowling *et al.*, 2010a;  
98 Magnuson *et al.*, 2012; Meyuhas and Drazzen, 2009; Thoreen, 2017). First, their kinetics of  
99 phosphorylation do not last the same and their different action in the regulation of protein synthesis  
100 machinery, which involves additional TOR targets, made it complex to decipher (Dowling *et al.*,  
101 2010a; Magnuson *et al.*, 2012; Meyuhas, 2015; Thoreen, 2017). As such, the TORC1 target LARP1  
102 (La-Related Protein 1) is a translation repressor that, according to a recent model, binds to the 5' end  
103 of mRNAs and thus competes with the translation initiation complex eIF4F (including eIF4E) and to  
104 some extent with S6K1 (Philippe *et al.*, 2018). TORC1 controls protein turnover through regulating  
105 UPS (Ubiquitin Proteasome System)- and UPS targeted-proteins abundance (Rousseau and Bertolotti,  
106 2016; Zhao *et al.*, 2016) as well as by canonical autophagy induction through regulating activity of the  
107 kinases ULK1 and 2/ATG1 (Human Uncoordinated-51-like autophagy activating kinase 1 and 2/yeast  
108 AuTophaGy related 1) (Velazquez and Jackson, 2018; Zhao *et al.*, 2015). Remarkably, due to  
109 reversible control of ULK1 by mTOR and AMP-activated Protein Kinase (AMPK) that senses low  
110 energy levels, mammalian growth homeostasis is orchestrated through dynamic signaling interplay of  
111 the triad of kinases, AMPK-TOR-ULK1. Under low energy, if ULK1 is activated through  
112 phosphorylation by AMPK, it can be impeded by TORC1 and in turn, ULK1-mediated  
113 phosphorylation can decrease activity of AMPK, establishing a negative feedback loop targeting the  
114 AMPK-mTOR signaling axis (Dunlop and Tee, 2013; Luo *et al.*, 2015). Also, a positive regulation  
115 loop occurs through phosphorylation by TOR and ULK1 of a component in autophagosome formation,  
116 which joins regulation of effectors by phosphorylation to regulation by ubiquitylation (Nazio *et al.*,  
117 2013).

118 More extensively, the crosstalk between different branches of the TOR network is nowadays  
119 upgraded by the emerging view that negative feedback loops where downstream targets become  
120 upstream regulators might be critical in the TOR pathway (Eltschinger and Loewith, 2016). As such,

121 the negative feedback loop of the TOR-S6K-IRS1 (Insulin Receptor Substrate 1) axis in response to  
122 TORC1 activation that is mediated by S6K1 attenuates PI3K-AKT signaling by phosphorylating IRS1  
123 and RICTOR leading to AKT kinase inhibition. These two examples of feedback loops state the  
124 importance of characterizing cell developmental or metabolic status when deciphering the role of  
125 specific TOR pathway effectors as physiology “customizes” TOR signaling backbone status. For  
126 instance, in the field of TOR-driven aging, cell entry into senescence is decelerated by rapamycin,  
127 preventing irreversible loss of proliferation capacity through inhibiting the senescence-associated  
128 secretory phenotype of cells without affecting cell cycle arrest (Wang *et al.*, 2017). This led defining  
129 new concepts and so new terms in order to delineate clear-cut functions of effectors in cell cycle arrest  
130 and/or senescence (Blagosklonny, 2012). Another important feature is that TOR basic targets S6Ks,  
131 4E-BPs, ULK1 or components of TOR complexes (SIN1, RAPTOR, RICTOR) very often carry  
132 multiple phosphorylation sites, which likewise helps connecting different signaling pathways to the  
133 TOR pathway to maintain cell homeostasis but makes analysis more complex (Batool *et al.*, 2017;  
134 Meyuhas, 2015; Tavares *et al.*, 2015). At last but not least, the recent discovery of new TOR  
135 complexes that do not contain RAPTOR or RICTOR reveals the extent of TOR function. As such, a  
136 complex TOR-RanBP2 (Ran Binding Protein 2) that ensures dynamic flux of nuclear import of  
137 ribosomal proteins (Kazyken *et al.*, 2014), a complex TOR-GIT1 (G-protein-coupled receptor kinase-  
138 interacting protein 1) essential for astrocyte survival (Smithson and Gutmann, 2016), a rapamycin  
139 insensitive TORC3 including at least LST8 and an unknown protein phosphorylating mSIN1 (Luo *et al.*,  
140 *et al.*, 2015), or a new rapamycin sensitive TORC acting on mRNA translation (Meyuhas, 2015) have  
141 been identified. Another cytoplasmic TORC3 activated in cancer solely contains mTOR, 4E-BP1 and  
142 the transcription factor ETV7 (leukemia virus E26 Transformation-specific Variant 7) but not the  
143 TORC1/2 crucial components LST8, RAPTOR, RICTOR or SIN1 (Harwood *et al.*, 2018). TOR  
144 complexes can have various intracellular localization, close to either the nucleus or the perinuclear  
145 region, lysosomes, mitochondria-associated endoplasmic reticulum membranes or plasma membrane

146 depending on nutrient status (Betz and Hall, 2013; Jhanwar-Uniyal *et al.*, 2017). This also holds true  
147 for the target S6K (Tavares *et al.*, 2015) and altogether this makes TORCs eclectic, in coherence with  
148 the role of TOR in cell growth homeostasis. Altogether, the discovery of new TORCs, their diverse  
149 intracellular localization, the interaction of TOR- and other- signaling pathways and the multiple  
150 phosphorylation sites of TOR pathway components reflect the deployment of TOR signaling and the  
151 importance to decipher its role in clearly defined cellular contexts.

152 In plants, early studies benefited from the conservation of TOR among species and the libraries  
153 of Arabidopsis insertion mutants which helped find knock-out mutants of homologs of yeast and  
154 mammalian genes encoding basic members of TORCs. Thus, Arabidopsis genome contains one *TOR*  
155 gene (*AtTOR*) (Menand-2002), two *RAPTOR* genes (Anderson *et al.*, 2005; Deprost *et al.*, 2005;  
156 Mahfouz *et al.*, 2006; Rexin *et al.*, 2015; Salem *et al.*, 2018) and two *LST8* genes (Moreau *et al.*,  
157 2012). Arabidopsis *raptor* mutants are still under study and sporadic embryonic arrest has been  
158 controversial likely due to poor quality of some insertion mutants (Rexin *et al.*, 2015), making it  
159 different from mammals where *RAPTOR* ablation is associated with male sterility (Xiong *et al.*,  
160 2017b). LST8s function is still in progress since only *lst8-1* mutant phenotype and not *lst8-2* is  
161 documented, yet altered growth and particularly metabolomic phenotype of *lst8-1* reminds amino acid  
162 accumulation observed in yeast *lst8* mutants (Moreau *et al.*, 2012). Thus, in the absence of RICTOR  
163 homologs, only basic TORC1 is characterized in plants and algae until now (Dobrenel *et al.*, 2016a;  
164 Perez-Perez *et al.*, 2017; van Dam *et al.*, 2011). The main plant TOR targets include S6K1 and S6K2,  
165 which are both related to mammalian S6K1, and the PP2A (Protein Phosphatase 2A) regulatory  
166 subunit TAP46 (Ahn *et al.*, 2011; Henriques *et al.*, 2010; Mahfouz *et al.*, 2006; Xiong and Sheen,  
167 2012). TOR negatively regulates autophagy also in plants and green algae (Liu and Bassham, 2010;  
168 Perez-Perez *et al.*, 2010) and even though convergence of UPS and autophagy has been demonstrated  
169 in plants (Marshall *et al.*, 2015) TOR dependent regulation of UPS is still unknown. Strikingly, the  
170 catalytic subunit KIN10 of SnRK1 (Snf1-Related protein Kinase 1), the plant homolog of mammalian



171 AMPK/yeast SNF1, regulates autophagy through inhibiting TOR and SnRK1 is not regulated by the  
172 AMP/ATP ratio similarly to yeast SNF1 and contrarily to mammalian AMPK (Soto-Burgos and  
173 Bassham, 2017). The position of the ATG1/13 kinase complex in autophagy is also central in plants  
174 with four isoforms of the ATG1 kinase, two of its partner ATG13 (Suttangkakul *et al.*, 2011) and  
175 accessory ATG proteins such as ATG11 (Li and Vierstra, 2014) reported in Arabidopsis, yet ATG1  
176 phosphorylation by TOR has not been demonstrated (Wang *et al.*, 2018a). Thus to control autophagy  
177 in plants, TOR might target ATG13 to regulate ATG1 similarly to yeast (Kamada *et al.*, 2010) rather  
178 than regulating both ATG13 and ULK1 as in mammals (Kim *et al.*, 2011). Interestingly, Arabidopsis  
179 ATG1 has a dual role through acting as a regulator and as a substrate of autophagy, likely a particular  
180 feature of plants (Bassham, 2009; Suttangkakul *et al.*, 2011). However, as plant autophagy effectors  
181 and processes are still under study (Masclaux-Daubresse *et al.*, 2017; Wang *et al.*, 2018a), this field  
182 requires more investigation. Other plant TOR targets were also identified (Shi *et al.*, 2018), including  
183 the transcription factors E2FA and E2FB which phosphorylation *in vitro* is lost by treatment with  
184 ATP-competitive inhibitors (Torins, see below) (Li *et al.*, 2017; Xiong *et al.*, 2013), or the hormone  
185 abscisic acid-receptor PYL1 (PYrabactin resistance 1-Like 1), which activity is associated with stress  
186 and senescence (Wang *et al.*, 2018b). In the absence of plant homologs of 4E-BP1, the axis TORC1-  
187 S6Ks is nowadays the most studied link between TOR and translation in plants, mainly through read  
188 out of ribosomal protein S6 phosphorylation (Dobrenel *et al.*, 2016b; Mahfouz *et al.*, 2006; Xiong and  
189 Sheen, 2012). However, the recent discovery of a Conserved Binding of eif4E1 (CBE1) plant protein  
190 (Patrick *et al.*, 2018) opens new possibilities of link between TOR and translation initiation in plants.  
191 In *Chlamydomonas reinhardtii*, recent phosphoproteomic studies identified TOR-inhibition dependent  
192 phosphorylation of proteins including ATG7, S6K, the ribosomal protein S6 and LARP1 (Roustan and  
193 Weckwerth, 2018; Werth *et al.*, 2018) showing conservation of effectors in algae and opening new  
194 avenues of TOR pathway characterization. In the red alga *Cyanidioschyzon merolae*, a  
195 phosphoproteomic analysis with the a transgenic strain overexpressing yeast FKBP12 identified

196 GLG1, an authentic GLycoGenin which phosphorylation is cancelled by rapamycin (Pancha *et al.*,  
197 2018). As systems biology and omics now start connecting TOR pathway with specific aspects of  
198 plant and algae physiology, new TOR targets could be discovered soon in photosynthetic organisms  
199 (Caldana *et al.*, 2013; Dobrenel *et al.*, 2016a; Mubeen *et al.*, 2018). New TOR complexes might exist  
200 in plants and algae, as discovered in animals, but their future identification would need more specific  
201 biochemical or genetic studies. Altogether, these data show that the TOR pathway includes conserved  
202 and specific effectors in photosynthetic organisms and thus its study benefits from outcomes from  
203 yeast and mammalian studies, as well as from plant and/or algae specific investigations.

204

### 205 **Rapamycin-FKBP12 -TOR inhibition in plants and algae: not an easy game**

206 In algal species, rapamycin sensitivity is species-dependent and growth inhibition level (GI %) and  
207 doses (nM) are highly variable as they range from (40%; 100 nM) for *Chlamydomonas reinhardtii*  
208 (Crespo *et al.*, 2005), to (40%; 50,000 nM) for *Euglena gracilis* (Mukaida *et al.*, 2016), to (slight  
209 effect; 10,000 nM) for the diatom *Phaeodactylum tricornutum* (Prioretti *et al.*, 2017) up to (0%; 1,000  
210 nM) for the red algae *C. merolae* (Imamura *et al.*, 2013). However, chlorophyll content decreased in *E.*  
211 *gracilis* and *C. merolae* from 1,000 nM but not in *C. reinhardtii* (Mukaida *et al.*, 2016). A rapamycin  
212 resistant FKBP12 loss-of-function mutant in *C. reinhardtii* allowed to demonstrate that rapamycin  
213 inhibits proliferation via the rapamycin-FKBP12 interaction, a strong argument for the further use of  
214 rapamycin in this alga (Crespo *et al.*, 2005). These few data show that rapamycin is not a general  
215 potent TOR inhibitor in algae species, reminding the variety of background responses of mammalian  
216 cell lines. In vascular plants, rapamycin hardly inhibits growth of various genera including  
217 *Arabidopsis*, *Nicotiana*, cotton or potato plantlets with some peculiar cases like tomato where partial  
218 growth inhibition has been observed (Deng *et al.*, 2017; Deng *et al.*, 2016; Mahfouz *et al.*, 2006;  
219 Menand *et al.*, 2002; Montane and Menand, 2013; Ren *et al.*, 2012; Song *et al.*, 2017; Sormani *et al.*,  
220 2007; Xiong *et al.*, 2016). Insensitivity or weak sensitivity to rapamycin has been attributed to low

221 ability of plant FKBP12 proteins to form the inhibitory ternary complex with rapamycin due to lack of  
222 conservation of aminoacid residues critical for interaction with rapamycin (**Supplementary Fig. S1**)  
223 (Choi *et al.*, 1996; Sormani *et al.*, 2007; Xu *et al.*, 1998). A similar situation was described in red algae  
224 (Imamura *et al.*, 2013). There is no straightforward evolutionary explanation for this particular feature  
225 of FKBP12s of plants and some algae, but we could speculate the selection of new FKBP12  
226 endogenous peptidyl-prolyl isomerase functions or a selective advantage to resist to the soil  
227 Streptomyces that produces rapamycin (Vezina *et al.*, 1975). Neither the yeast two-hybrid analysis  
228 nor an *in vitro* interaction assay could demonstrate a rapamycin-dependent interaction between  
229 Arabidopsis FKBP12 (AtFKBP12) and the AtTOR-FRB domain but this domain was able to form a  
230 complex with human (Hs) or yeast FKBP12 (Mahfouz *et al.*, 2006; Menand *et al.*, 2002; Sormani *et*  
231 *al.*, 2007). As a consequence, Arabidopsis plants could be made sensitive to rapamycin by  
232 overexpression of yeast or human FKBP12 (Deng *et al.*, 2016; Leiber *et al.*, 2010; Ren *et al.*, 2012;  
233 Sormani *et al.*, 2007; Xiong and Sheen, 2012). A yeast-FKBP12 overexpressing line in the red alga *C.*  
234 *merolae* similarly confers sensitivity to 10-500 nM rapamycin (Imamura *et al.*, 2013). However, to our  
235 opinion, the dogma of plant TOR kinase inhibition by rapamycin through transgenic FKBP12  
236 overexpression deserves little bit more attention.

237         Several groups reported that Arabidopsis seedlings grown on solid media are insensitive to  
238 rapamycin up to ca. 10  $\mu$ M (Deng *et al.*, 2016; Mahfouz *et al.*, 2006; Ren *et al.*, 2012; Sormani *et al.*,  
239 2007). Such concentration range is 100-1000 times the concentration that inhibits proliferation of yeast  
240 (100 nM block cells in G1 with large unbudded cells as the terminal phenotype (Heitman *et al.*, 1991)  
241 or that reduces cell size and proliferation of lymphocytes B cells (EC50 0.005-0.5 nM and maximal  
242 inhibition of ca. 50-70% up to 100 nM) or of mouse embryonic fibroblasts (50-250 nM) (Sarbasov *et*  
243 *al.*, 2006; Thoreen and Sabatini, 2009; Wicker *et al.*, 1990). Later, AtTOR-dependent phosphorylation  
244 at P-T449 (equivalent to T389 in animal) of AtS6K1 overproduced in transfected protoplasts was  
245 found inhibited by far much lower concentrations of rapamycin when FKBP12 was co-expressed

246 compared to the AtS6K alone. Indeed, 100 to 1000 times lower concentration of rapamycin was  
247 needed to erase S6K1 P-T449 when S6K1 was overexpressed in combination with either AtFKBP12  
248 or HsFKBP12 respectively (Xiong and Sheen, 2012). This showed that a high amount of HsFKBP12  
249 “optimize” the titration of rapamycin to inhibit plant TOR. Thus, the affinity to rapamycin and the  
250 stoichiometry of each component of the ternary complex might influence the stability of TOR complex  
251 conformation shift and therefore the outcome of TOR inhibition. In other words, the poorest the  
252 interaction FKBP12-rapamycin is, the highest the rapamycin concentration is required to erase  
253 AtS6K1 P-T449. Thus, if we consider that this is the rule despite the peculiar physiological context of  
254 protoplasts incubated in mannitol and KCl in which it has been studied (no nutrients), a low amount of  
255 AtFKBP12 together with a poor binding of endogenous AtFKBP12 to AtTOR can explain why plants  
256 are poorly sensitive to rapamycin. Deng *et al.* similarly developed transgenic plants overexpressing  
257 *FKBP12* coming from Arabidopsis, yeast and human but showed that AtFKBP12 overexpression  
258 could not make plants sensitive to rapamycin (**Fig. 1C**) (Deng *et al.*, 2016). This discrepancy with the  
259 data of Xiong and Sheen (Xiong and Sheen, 2012), shows the possible drawback of building  
260 transgenic lines to overexpress FKBP12 (FKBP12<sup>OX</sup>). Yet, the overexpressed yeast FKBP12 was more  
261 efficient than HsFKBP12 to increase plant sensitivity to rapamycin (**Fig. 1C**) (Deng *et al.*, 2016).

262 Growth conditions also influence rapamycin sensitivity as Arabidopsis seedlings grown in  
263 liquid culture were reported sensitive to rapamycin (Deng *et al.*, 2016; Xiong *et al.*, 2013; Xiong and  
264 Sheen, 2012). An interesting explanation for the discrepancy between plants grown on liquid and solid  
265 media was proposed by M Ren and colleagues who suggested that a hypoxia stress could facilitate  
266 rapamycin action in Arabidopsis (Deng *et al.*, 2016). Indeed, growth is dramatically slowed down in  
267 liquid media as after 9 days, seedlings roots were around 2-3 cm long (Xiong and Sheen, 2012), which  
268 is around 2-3 times less than vertically grown seedlings on solidified medium (Montane and Menand,  
269 2013; Ren *et al.*, 2012). It is therefore likely that hypoxic stress might upregulate *AtFKBP12*  
270 expression as FKBP12s are reported to have a role in stress response (Dong *et al.*, 2018; Geisler and

271 Bailly, 2007) and/or that AtTOR is largely inhibited in this condition which might make AtTOR  
272 activity easier to be inhibited by rapamycin. Interestingly, the sensitivity of Arabidopsis seedlings to  
273 the rapamycin structurally related compound FK506 (that binds FKBP12 but not TOR), was tested in  
274 WT and yeast-FKBP12<sup>OX</sup> lines (Zhang *et al.*, 2013). The authors concluded that FK506 has no effect  
275 on seedlings growth but the seedling phenotype shown after 25 days on half strength Murashige and  
276 Skoog (MS) medium containing 20  $\mu$ M FK506 appears to us different from the control. Indeed, roots  
277 hardly grew in the solid medium but grew in the air outside the solid matrix, a feature already observed  
278 after 9 days on non-optimal full strength 1xMS medium (Ren *et al.*, 2012). This altered growth is  
279 characteristic of a stress due to non-optimal or to toxic drug containing medium and might reveal  
280 potential TOR-rapamycin-independent phenotypes. Thus, we think that more detailed analysis is  
281 required before ruling out that FK506 affects or not the physiology of yeast-FKBP12<sup>OX</sup> lines.

282         Additionally, Arabidopsis FKBP12 was shown to interact with a nuclear protein that controls  
283 endoreduplication and therefore might control this process as in mammals (Vespa *et al.*, 2004). As in  
284 the case of calcineurin, HsFKBP12 is also a subunit of the transforming growth factor B (TGF-  
285  $\beta$ ) type I receptor, a transmembrane Ser/Thr kinase that regulates cell growth and differentiation (Gold  
286 *et al.*, 1997). HsFKBP12<sup>-/-</sup> cells show cell cycle arrest due to impaired regulation of TGF- $\beta$  receptor  
287 signaling (Aghdasi *et al.*, 2001). Thus, it appears relevant to wonder whether in the absence of  
288 rapamycin AtFKBP12 is involved in cell cycle regulation or other signaling regulations that might  
289 interfere with TOR signaling studies with FKBP12<sup>OX</sup> lines. Indeed, expressing an heterologous  
290 *FKBP12* gene might also change plant physiology as the *PaFKBP12* gene from the Antarctic moss  
291 *Polytrichastrum alpinum* ectopically expressed in Arabidopsis increases plant stress tolerance (Alavilli  
292 *et al.*, 2018). Thus, even though FKBP12<sup>OX</sup> lines do not have a macroscopic phenotype (Deng *et al.*,  
293 2016; Sormani *et al.*, 2007), it does not preclude conditional cell/tissue responses compared to WT.  
294 Not least, when regarding the other component of the duo FKBP-Rapamycin, a concentration range of  
295 0.5-5  $\mu$ M rapamycin was shown to interfere with interactions of the core particle 20S with its cellular

296 activators and consequently to inhibit proteasome by attenuating major peptidase activities (Osmulski  
297 and Gaczynska, 2013). The authors hypothesized that interactions of the proteasome with rapamycin  
298 induce a maximal conformation shift of the proteasome, which results in compromised gating of  
299 substrates. Such an off target of rapamycin might interfere with other functions independently of the  
300 effects of TOR inhibition. Whether proteasome is an off target of rapamycin also in plants is unknown  
301 but should be carefully considered as proteolysis is enhanced following TOR inhibition (Zhao *et al.*,  
302 2015) and UPS and autophagy converge in Arabidopsis (Marshall *et al.*, 2015). At last, another hint is  
303 the putative role of metabolites interacting with TOR such as phosphatidic acid that might impede  
304 interaction rapamycin-TOR and explain the strong variation of rapamycin dose to inhibit TOR in  
305 different mammalian cell lines (Mukhopadhyay *et al.*, 2016). Anyhow, altogether this underscores that  
306 FKBP12 and/or rapamycin dosage as well as the cell physiology might be carefully controlled as they  
307 influence TOR inhibition-dependent results. This also opens the question of the selectivity of  
308 rapamycin. Therefore, compared to yeast and mammals, the conditional FKBP12 overexpression-  
309 dependent allosteric inhibition of TOR by rapamycin in plants might easily turn to be a conundrum.

310 Another aspect to underline is that for each combination of FKBP12<sup>OX</sup> lines, the dose response  
311 to rapamycin shows that growth cannot be completely inhibited. Seedlings growth of yeast-FKBP12<sup>OX</sup>  
312 lines was partially inhibited by 1-20  $\mu$ M rapamycin (Deng *et al.*, 2016; Ren *et al.*, 2012) or by even  
313 lower range of 10-100 nM (Zhang *et al.*, 2013). Anyhow, routine rapamycin concentration ranges are  
314 4-10  $\mu$ M to inhibit such lines (Leiber *et al.*, 2010; Sormani *et al.*, 2007; Xiong and Sheen, 2012),  
315 which makes it hard to appreciate rapamycin potency in plants. However, growth inhibition of yeast-  
316 FKBP12<sup>OX</sup> plants by rapamycin never exceeds a plateau value of ca. 50% (**Fig. 1C**), which reminds  
317 the incomplete efficacy of rapamycin action observed in mammals (**Fig. 1A and C**). Similarly, in the  
318 green algae *C. reinhardtii*, which is naturally sensitive to rapamycin (**Supplementary Fig.S1**) in both  
319 solid and liquid media, maximal growth inhibition was also ca. 50% (**Fig. 1E**) (Crespo *et al.*, 2005;  
320 Juppner *et al.*, 2018; Roustan and Weckwerth, 2018). Therefore, in both yeast-FKBP12<sup>OX</sup> plant lines

321 and rapamycin-sensitive WT algae challenged till now, full growth inhibition cannot be reached with  
322 rapamycin (**Fig. 1C and D**), making rapamycin efficacy not maximal. With that in mind, we can  
323 conclude that rapamycin can be used with more confidence in *Chlamydomonas reinhardtii* than in  
324 plants and other algae as, even if TOR inhibition is probably partial, transgenic over-expression of  
325 FKBP12s is avoided.

326

### 327 **Inhibition of TOR by ATP competitive inhibitors: a new deal**

328           Within this highly dynamic research field, studies of TOR kinase have considerably  
329 increased over the last 10 years and limitations of allosteric rapamycin- and rapalogs-based clinical  
330 strategies have pushed toward the development of orthosteric ATP-competitive mTOR inhibitors that  
331 were called asTORis (active site TOR inhibitors), TORKis or TKIs (TOR Kinase Inhibitor) (Martelli  
332 *et al.*, 2018). In contrast to rapamycin, they target the kinase domain of mTOR and are able to fully  
333 inhibit mammalian TORC1 activity in a dose dependent manner but also TORC2 and other TOR  
334 complexes (**Fig. 1A and B**) (Chresta *et al.*, 2012; Dowling *et al.*, 2010b; Harwood *et al.*, 2018; Kang  
335 *et al.*, 2013).

336           Here we would like to remind that the terms of potency, efficacy, selectivity, metabolic  
337 stability, off rate (also called associated residence time), and off targets as well as pharmacokinetic  
338 characteristics of a drug at the organism level (PK), altogether define a drug singularity. **Potency** and  
339 **efficacy** are parameters that are derived from graded dose-effect curves and that can be used to  
340 compare drugs that elicit the same pharmacological effect (Mosby's Medical Dictionary, 2009).  
341 **Potency**, which is a measure of the sensitivity of a target organ or tissue to a drug, is a relative term  
342 that relates the amount of one drug required to produce a desired level of effect to the amount of a  
343 different drug required to produce the same effect. On the semi-logarithmic graded dose-effect plot,  
344 the curve of the most potent agent tends to be in the left side of the graph and the median effective

345 concentration (EC50) is lower. A drug's potency is influenced by its affinity for its receptor and  
346 therefore independent of its maximal effect. *Efficacy* (or intrinsic activity) is the drug property that  
347 allows the receptor-bound drug to produce its pharmacological effect. The relative efficacy of two  
348 drugs that elicit the same effect can be measured by comparing the maximum effects of the drugs. A  
349 drug can have high potency but poor efficacy, meaning that the response is seen at very low doses and  
350 remains small even at high doses. This is the case of rapamycin which is a highly potent but poorly  
351 efficient TOR allosteric inhibitor (**Fig. 1A, C and E**) compared to active site TOR inhibitors which are  
352 highly efficient (**Fig. 1B, D and F**). If a drug has one effect, and only one effect on all biological  
353 systems it possesses the property of *specificity*. In experience, the vast majority of drugs are *selective*  
354 rather than *specific* (Davis *et al.*, 2011). “A drug with the appropriate balance of avoidance of  
355 undesirable targets (narrow selectivity) and coverage of one or more targets of interest (broad  
356 selectivity) is a continual drug development challenge. In many cases this objective is attained through  
357 trial and error, but there are rational approaches that can guide the tuning of selectivity, and examples  
358 have been published that illustrate a number of generalizable strategy” (Huggins *et al.*, 2012). Thus, “a  
359 Selectivity score (S) for each drug can be calculated by dividing the number of kinases found to bind  
360 with dissociation constant  $<3 \mu\text{M}$ ” (or sometimes  $10 \mu\text{M}$ ) “by the total number of distinct kinases  
361 tested. The selectivity score is an unbiased measure that enables quantitative comparisons between  
362 compounds and the detailed differentiation and analysis of interaction patterns” (Karaman *et al.*,  
363 2008).

364 At last, in pharmacology, an inhibiting or effective concentration (IC or EC) refers to a  
365 concentration of a drug that produces a biological response in case of enzymology *in vitro* assays or  
366 when unicellular organisms or mammalian cell cultures are tested. IC refers to an assay where there is  
367 decrease in activity whereas EC rather refers to a drug that activates a system. The term effective dose  
368 (ED) refers to *in vivo* studies when used in living organisms such as animals to usually determine the  
369 median effective dose (ED50) and/or the median lethal dose (LD50). Usually, in the context of studies



370 involving asTORis, potency values obtained by means of *in vitro* enzyme-based assays ( $IC_{in\ vitro}$ ) are  
371 generally different than potency values ( $IC_{cell}$ ) obtained by cell-based assays treating cells before  
372 measuring various enzymatic products (also called cell potency values) because many targets can be  
373 modified at more than one phosphorylation site or in more than one way, *e.g.*, ubiquitylation or  
374 acetylation (Carlson *et al.*, 2009). Dissecting biochemical effects using *in vitro* grown cell lines might  
375 also give rise to different EC values depending on the cell physiology/line and is a far much different  
376 task than looking for clinical outcomes. Indeed the efficient doses (ED) are usually higher than those  
377 of *in vitro* studies likely to encompass drawbacks linked to PK properties, metabolic stability and  
378 putative off targets effect in organisms. A tool such as KInhibition portal  
379 (<https://kinhibition.fredhutch.org>) might help choosing a set of selective drugs among thousands  
380 depending on the objective (Bello and Gujral, 2018). Thus, designing a scale of inhibitor “strength” of  
381 a set of inhibitors solely from enzymatic properties (*in vitro*  $IC_{50}$ ) to calibrate experiments with living  
382 cells or organisms might be hazardous (Michel and Seifert, 2015).

383         Chemical structure activity relationship through docking studies using the TOR kinase domain  
384 with the dual PI3K/PIKK inhibitor NVP-BEZ235 (BEZ235), the TOR selective inhibitor PP242, and  
385 the TOR specific inhibitor KU-0063794 showed that drugs in development utilize a novel  
386 pharmacophore space to achieve specificity of TOR inhibition (Sturgill and Hall, 2009). So around the  
387 year 2009, several compounds were reported as asTORis (**Fig. 2**): PP242 (Feldman *et al.*, 2009),  
388 Torin1 (Liu *et al.*, 2010; Thoreen *et al.*, 2009), KU-0063794 (Garcia-Martinez *et al.*, 2009), WYE-354,  
389 600 and 687 (Yu *et al.*, 2009) and others reviewed by Benjamin and colleagues (Benjamin *et al.*,  
390 2011). These compounds were generally developed from dual PI3K/PIKK inhibitors or inhibitors more  
391 largely involved in the PI3K/AKT axis and have different core structure (Andrs *et al.*, 2015; Garcia-  
392 Echeverria, 2011; Liu *et al.*, 2012a). They were TOR selective, having  $IC_{50}$  for TOR lower than for  
393 PI3Ks and also for other PIKKs (Benjamin *et al.*, 2011). For instance, Torin1 efficiency towards TOR  
394 was compared to the effect of the dual PI3K/PIKK inhibitors PI103 and BEZ235 and its high

395 selectivity towards a panel of kinases including PI3Ks and PIKKs was shown. Torin1 has a quinolone  
396 core structure expected to share the same binding mode as BEZ235 with mTOR, PI3K and other PIKK  
397 family members while PP242 has a pyrazolopyrimidine core structure derived from PP2 (Feldman *et*  
398 *al.*, 2009). KU-0063794 and WYE-354 also derive from other dual PI3K/PKK inhibitors such as PI-  
399 103 and LY294002 and contain a morpholino-substituted heterocycle. These asTORis were reported  
400 more efficient than rapamycin by measuring their capacity to phosphorylate TOR targets and also  
401 inhibit cell proliferation more efficiently. So new TORC1-dependent functions and previously found  
402 rapamycin resistant were deciphered due to higher efficacy of asTORis (Feldman *et al.*, 2009; Thoreen  
403 and Sabatini, 2009) leading to new advances in TOR pathway knowledge (Guertin and Sabatini,  
404 2009). For instance, the more effective TOR inhibition unveiled rapamycin resistant levels of  
405 regulation in cap-dependent initiation of translation, protein synthesis and proliferation (Dowling *et*  
406 *al.*, 2010b). This also helped show that mTOR activates cap-dependent translation of cyclins and  
407 represses cap-independent translation of p27/KIP1, an inhibitor of CDK (Cyclin Dependent Kinase),  
408 therefore activating cell proliferation (Thoreen *et al.*, 2009). These differential effects of rapamycin on  
409 substrates phosphorylation compared to that of the ATP competitive inhibitor Torin1 were studied  
410 through designing peptides from well-known TORC1 targets containing phosphorylation sites (Kang  
411 *et al.*, 2013). When Torin1 blocks the phosphorylation of all TORC1 dependent phosphorylated sites  
412 in all TOR protein targets, some are not dephosphorylated by rapamycin (called strong target) and  
413 some are (called poor target). This concept of substrate quality is a property of TOR effector sites,  
414 which can explain that their differential phosphorylation vary with growth conditions. Furthermore,  
415 poor and strong targets can be found in the same protein (**Fig. 3**). In parallel, strong targets were also  
416 found phosphorylated in cells growing under partially depleted nutrient conditions (**Fig. 3**). Hence,  
417 rapamycin can be highly potent and selective for some poor mTOR targets such as S6K T389 and 4E-  
418 BP1 S65, but its intrinsic activity or efficacy cannot be maximal since TOR is still able to  
419 phosphorylate the strong mTORC1 targets such as 4E-BP1 T37/46 and ULK1 S758 in presence of

420 rapamycin (Kang *et al.*, 2013). Therefore, due to the incomplete intrinsic efficacy, rapamycin-  
421 dependent TOR inhibition by an acute dose might lead to error-prone interpretation of data especially  
422 when targets are not well identified and/or when a chronic dose of rapamycin is applied (Sarbasov *et*  
423 *al.*, 2006).

424 A comparison of selectivity, potency and metabolic stability of four asTORis mentioned above  
425 carrying different structure, *i.e.*, Torin1, PP242, WYE-354 and KU-0063794 (**Fig. 2**) was reported and  
426 it appears to us to be a good example of how selectivity of a drug to TOR is demonstrated through  
427 tests involving many different assays (Liu *et al.*, 2012a). They all exhibited highly potent and similar  
428 IC50 values against the recombinant mTOR kinase domain but their relative cellular potency EC50  
429 against the TORC1 complex was: Torin1 > KU-63794 > WYE-354 > PP242. Their relative selectivity  
430 score toward a panel of 442 kinases was: KU-0063794 > WYE-354 > Torin1 > PP242. Other kinase  
431 assays showed that Torin1 concentration above 1  $\mu$ M was able to inhibit the other PIKKs: ATM, ATR  
432 and DNA-PK. The metabolic stability was also better with KU-0063794 and WYE-354 than with  
433 Torin1 and PP242. However, Torin1 had a slower off-rate as the duration of S6K1 (pS6K-Thr-389)  
434 and PI3K-dependent (p-AKT-Thr-308) phosphorylation last 16 hours *vs* 1 hour for the 3 other drugs  
435 after extensive washing out the drug. Altogether, authors' conclusion of the study of these four  
436 asTORis was to avoid PP242 and to cautiously interpret data when Torin1, KU-0063794 and WYE354  
437 are used at concentrations above 1  $\mu$ M. Proliferation assays on mouse embryonic fibroblasts (MEFs)  
438 showed that in the range of 10-500 nM, rapamycin induces a plateau value of ca. 50-60% inhibition  
439 without any dose dependence, whereas Torin1 induces 40% to 100 % inhibition between 10 and 250  
440 nM when IC50 kinase values were 1-10 nM (Thoreen *et al.*, 2009). Hence, **Table 1** shows that IC50  
441 values for *in vitro* TOR kinase activity do not fully predict the IC50 of proliferation. These differences  
442 likely deal with intrinsic PK, metabolic stability of the drug, or posttranslational modifications of the  
443 target as well as drug efflux or inactivation by cells as in yeast (Liu *et al.*, 2012b).

444 Improvement of the pharmacokinetic properties of KU-0063794 led to the development of

445 AZD-8055, which is very highly selective for mTOR over PI3Ks and PIKKs (Chresta *et al.*, 2012;  
446 Garcia-Echeverria, 2011; Marshall *et al.*, 2011) and more recently to the less selective but more stable  
447 sister compound AZD2014 (Pike *et al.*, 2013) (**Fig. 2**), both used in clinical trials (Garcia-Echeverria,  
448 2011). A continuous development of intermediate compounds of this series that showed very high  
449 specificity towards PI3Ks although lower potency towards TOR led to the design of AZD3147, a new  
450 highly selective inhibitor of TORC1 and TORC2 (Pike *et al.*, 2015) that can circumvent the  
451 discontinuity of clinical trials (Martelli *et al.*, 2018). Similarly, other derived compounds were  
452 developed in parallel like WYE-125132 (WYE-132) or Torin2 (Liu *et al.*, 2013; Yu *et al.*, 2010).  
453 WYE-132 has better pharmacokinetics properties than WYE-354 and is highly selective for mTOR  
454 over PI3K and the PIKK ATR (Yu *et al.*, 2010). Torin2 has improved pharmacological and solubility  
455 properties compared to its structural analogue Torin1 but also significant activity against mTOR,  
456 ATM, ATR, and DNA-PK, as well as both *in vitro* and *in vivo* antitumor efficacy, being therefore a  
457 potent broadly active pan-PIKK kinase inhibitor (Liu *et al.*, 2013). Indeed, if Torin2 most potently  
458 inhibits mTORC1 and mTORC2 *in vivo* at concentrations of less than 10 nM, it also inhibits ATR,  
459 ATM, and DNA-PK at concentrations between 20 and 100 nM and PI3K at concentrations above 200  
460 nM. This is in contrast with Torin1 which only exhibits moderate inhibition of DNA-PK (250 nM) but  
461 is inactive against other PIKK-family kinases. Unexpectedly, Torin2 also has a lower residence time  
462 than Torin1 (4h vs 16h) leading to suggest that Torins might induce a TOR conformation change in the  
463 kinase that is energetically difficult to recover from rather than different binding affinities. This adds a  
464 specific feature to Torins that distinguish them from other asTORis, showing that Torin1 and Torin2  
465 although structurally close cannot only be compared in terms of potency. Moreover, the intentional  
466 development of new dual Torin2 analogs that inhibit both mTOR and ATR (Shaik *et al.*, 2018) for  
467 clinical purposes shows that driving drug selectivity to specificity is a difficult chemistry task.  
468 Therefore, the crosstalk between TOR pathway and other pathways such as the DNA damage response  
469 (Li *et al.*, 2012; Silvera *et al.*, 2017) involving other PIKK close to TOR might hamper the discovery

470 of true TOR targets with Torin2 and Torin2 analogs. In addition, Torin2 was reported as an  
471 antimalarial agent 1,000-fold selective to malaria parasites over mammalian cells whereas TOR  
472 homolog is not found in *Plasmodium falciparum* (Hanson *et al.*, 2013). Therefore, Torin2 is  
473 particularly known to target other eukaryotic proteins than TOR and should be used with caution for  
474 biological studies.

475         Nowadays, the use of more than one selective and potent inhibitor through targeting for  
476 instance two pathways or sub-pathways is more and more explored in medicine rather than using dual  
477 PI3K/mTOR inhibitors of a single pathway, which can have the “possible drawback of association  
478 with greater toxicity” (Simioni *et al.*, 2014). Furthermore, novel compounds are continuously searched  
479 to circumvent the discontinuity of potent and selective compounds yet cytostatic or unstable in clinical  
480 trials (Andrs *et al.*, 2015; Chen *et al.*, 2012; Estrada *et al.*, 2013; Fraser *et al.*, 2016; Mortensen *et al.*,  
481 2015; Nowak *et al.*, 2009; Park *et al.*, 2014; Pei *et al.*, 2012; Slotkin *et al.*, 2015; Walters and Cox,  
482 2018; Zheng and Jiang, 2015). Thus, although selectivity is not always the major criteria in clinical  
483 trials, it is an essential criteria for the choice of an inhibitor to elucidate the role of a particular kinase  
484 in biological tissues and *in vitro* studies (Arrowsmith *et al.*, 2015). This illustrates why potency and  
485 efficacy of selective inhibitors should be carefully examined and that the concept of inhibitor strength  
486 can be misleading according to experts (Michel and Seifert, 2015). This also underlines that  
487 testing/using more than one TOR ATP competitive inhibitor should help identifying and confirming  
488 TOR-dependent regulated functions.

489

### 490 **ATP-competitive TOR inhibitors in plant and algae**

491 The first asTORis that have been used in plants were among those presented above, *i.e.*, KU-0063794,  
492 AZD-8055, Torin1 and Torin2, WYE-354 and WYE-132. It was remarkable that KU-0063794 and  
493 WYE-354 and their improved derived molecules, AZD-8055 and WYE-132, followed the same  
494 relative potency than in mammalian cells, *i.e.*, AZD-8055 > KU-0063794 and WYE-132 > WYE354

495 (Table 1) (Montane and Menand, 2013). We encountered solubility problems with Torin1 in our  
496 culture conditions, and among the drugs we could get and test at that time; AZD-8055 (Fig. 1D) and  
497 WYE-132 quickly became our favorites to fully inhibit Arabidopsis WT seedlings growth because of  
498 high reproducibility of responses and of temporal stability of dose-dependent inhibition. Furthermore,  
499 we demonstrated that the plant growth inhibition by AZD-8055 and WYE-132 is TOR-dependent by  
500 showing induced haploinsufficiency to TOR as the *TOR/tor* heterozygote mutants are hypersensitive to  
501 both inhibitors compared to WT (Fig. 1D) (Montane and Menand, 2013). Maximal efficacy was  
502 shown through dose-response curves showing complete inhibition of root growth, as opposed to  
503 rapamycin in plants or algae (Fig. 1C-F). asTORis were potent in most photosynthetic eukaryotes as  
504 they strongly inhibit growth of a large variety of plants (Arabidopsis, potato, tomato, rice; *Lotus*,  
505 millet, *Nicotiana*), and proliferation in both green algae and diatoms (*C. reinhardtii*, *P. tricornutum*)  
506 (Deng *et al.*, 2017; Dong *et al.*, 2015; Dong *et al.*, 2018; Imamura *et al.*, 2016; Montane and Menand,  
507 2013; Prioretti *et al.*, 2017; Song *et al.*, 2017). Table 1 and Fig. 2 show the concentration range of the  
508 main asTORis used to inhibit proliferation, to study TOR functions and that helped find new TOR  
509 targets in photosynthetic eukaryotes: KU-0063794, AZD-8055, Torin1 and Torin2 (Deng *et al.*, 2017;  
510 Dong *et al.*, 2015; Kravchenko *et al.*, 2015; Li *et al.*, 2015; Li *et al.*, 2017; Mohammed *et al.*, 2018;  
511 Montane and Menand, 2013; Mubeen *et al.*, 2018; Ouibrahim *et al.*, 2015; Pfeiffer *et al.*, 2016;  
512 Prioretti *et al.*, 2017; Schepetilnikov *et al.*, 2013; Schepetilnikov *et al.*, 2011; Schepetilnikov *et al.*,  
513 2017; Wang *et al.*, 2018b; Werth *et al.*, 2018). Some authors tried to establish a scale of “strength” of  
514 these inhibitors in plants starting from values of IC50 kinase *in vitro* (Deng *et al.*, 2016; Xiong *et al.*,  
515 2017a). Here, as we have discussed above, we would like to stress that knowledge from the  
516 biochemical and animal fields should be taken in consideration when using such inhibitors. Indeed, as  
517 shown above for KU-0063794, Torin1 and AZD-8055 (Table 1), using the IC50 of *in vitro* kinase to  
518 predict IC50 of proliferation hardly works in mammals and plants. This might hide other  
519 characteristics of a drug *in vivo* as well as its speciation in culture medium. As reported above, the

520 potency of Torin1 in animal cells was higher than that of KU-0063794 but the selectivity was the  
521 opposite. Regarding the use of Torins and in particular Torin2 as an asTORi, we have mentioned  
522 above that it was described as a pan-PIKK family inhibitor as it exhibited potent biochemical and  
523 cellular activity against PIKKs, including ATM and ATR (Liu *et al.*, 2013). Indeed, mammalian  
524 mTOR is involved in the network of responses between DNA damage and cell cycle control, including  
525 the activity of the ATM/ATR-CHK1/CHK2-p53 axis (Silvera *et al.*, 2017). Since ATM and ATR are  
526 conserved in plants and algae and regarding the known role of Arabidopsis ATM in regulation of  
527 meristem activity and DNA-damage responses (Fulcher and Sablowski, 2009; Hisanaga *et al.*, 2013;  
528 Ricaud *et al.*, 2007), care should be taken when analyzing data with Torin2 in plants, especially when  
529 high concentrations are used as mentioned above. For our part, we therefore avoid using Torins in  
530 order to selectively inhibit the TOR pathway and checked that selective ATP competitive ATM  
531 inhibitors did not inhibit growth and so could be used as a selectivity control toward TOR inhibitors  
532 (Montane and Menand, 2013). The specificity of a cellular response towards TOR inhibition can also  
533 be confirmed in plant and algae through comparing the effect of different selective asTORis such as  
534 AZD-8055 and WYE-132 (Barrada *et al.*, 2019; Prioretti *et al.*, 2017) (Prioretti *et al.*, 2017)but it has  
535 to be done at doses leading to similar growth inhibition. Furthermore, we should keep in mind that we  
536 should not expect identical molecular and cellular effects of rapamycin and asTORis in plants and  
537 algae as rapamycin does not inhibit all TORC1 activity, and might also not inhibit other potential new  
538 plant/algae TOR complexes (see above). Taking into account the conservation of the minimal core  
539 TORC1 in plants, and the specificity of plant targets that only start to emerge, we think that other plant  
540 TOR target sites than the sole S6K T449 will need to be developed to accurately record the level of  
541 plant TOR inhibition. Indeed, the mammalian S6K1 T389 equivalent of plant S6K T449 corresponds  
542 to a poor TOR target (Kang *et al.*, 2013).

543

## 544 **Pharmacogenetic screens in plant and algae**

545 Selective active site inhibitors have several advantages to other methods of kinase inhibition  
546 for genetic screens. First, they allow avoiding possible drawbacks associated with transgenic lines.  
547 Second, they allow to control the time of and the level of inhibition on a given organism, organ, tissue  
548 or cell by following the kinetics of any measurable parameter and its dose-dependent evolution, an  
549 important aspect in case of essential genes like *TOR*. Inhibiting TOR from a known WT physiological  
550 context is definitely different than comparing loss of function or overexpressing functions.  
551 Furthermore, being cytostatic also in plants (Montane and Menand, 2013), these inhibitors offer the  
552 possibility to rescue hypersensitive plants and to more accurately follow reciprocal changes or point  
553 out any differential behavior of targets when inhibition is relaxed. To date, few screens of mutants  
554 resistant or hypersensitive to TOR inhibitors have been reported in plants and algae yet they revealed  
555 important aspect of TOR signaling (Barrada *et al.*, 2019; Couso *et al.*, 2016; Crespo *et al.*, 2005; Li *et*  
556 *al.*, 2015). Recently, the *vip1-1 C. reinhardtii* mutant hypersensitive to the first saturating  
557 concentration of 500 nM rapamycin inhibiting growth to the 50% plateau value was isolated (Couso *et*  
558 *al.*, 2016). This mutant which was also hypersensitive to the single concentration tested of 500 nM  
559 AZD-8055 or Torin1 revealed an interaction between TOR and inositol polyphosphate intracellular  
560 signaling. In Arabidopsis, a first screen of mutants that show no chlorosis of cotyledons induced by 2  
561  $\mu$ M AZD-8055 allowed to select 9 mutants among which a new *ABI4* (*ABA-INSENSITIVE 4*) allele,  
562 revealing a new role of TOR in abscisic acid (ABA) signaling (Li *et al.*, 2015). Interestingly, in our  
563 culture conditions we never observed AZD-8055-induced chlorosis at doses up to 10  $\mu$ M (Montane  
564 and Menand, 2013), meaning that growth conditions have to be considered consistently with the role  
565 of TOR in response to nutrients and stress. We recently screened EMS Arabidopsis mutants resistant  
566 to a concentration of 1 $\mu$ M AZD-8055 inhibiting 90% of root lengthening and discovered that the  
567 homolog of yeast YAK1 (Yet Another Kinase 1) and human DYRK1A (Dual Specificity Tyrosine  
568 Phosphorylation Regulated Kinase 1A) was a TOR-inhibition dependent downstream repressor of



569 plant cell proliferation (Barrada *et al.*, 2019). We also showed that pINDY, an-ATP competitive  
570 inhibitor of DYRK1A, mimics Arabidopsis *yak1* loss-of-function mutations. This offers a new way to  
571 study TOR-YAK1 axis in plants. In addition, we isolated other mutants sensitive or resistant to AZD-  
572 8055 concentrations leading to other levels of growth inhibition which are now under study. Hence,  
573 together with studies of known mutants of components of the plant/algae TORC1 pathway, new  
574 screening approaches might actively help deciphering TOR pathways in photosynthetic organisms.

575 In addition to being proof-of-concept that the pharmacogenetic screens can help identify new  
576 functions of TOR in plant and algae, these studies remind the importance to carefully design the  
577 conditions of the screen but also of further studies. For instance, the haploinsufficiency phenotype of  
578 *TOR/tor* heterozygotes discussed above is a nice illustration of the importance of the “right” dose of an  
579 inhibitor to compare physiological context of two genetic backgrounds (Montane and Menand, 2013).  
580 The *TOR/tor* heterozygotes were clearly hypersensitive to AZD-8055 concentrations between 0.1 and  
581 1  $\mu\text{M}$ , but grow the same as the WT at doses below 0.03  $\mu\text{M}$  and above the maximal inhibitory  
582 concentrations of 3 $\mu\text{M}$ , that almost completely inhibit root growth (**Fig. 1 D**). For instance, to compare  
583 YAK1- and TOR- expression patterns by GUS staining in roots which growth was similarly inhibited  
584 by AZD-8055, we used twice lower AZD-8055 concentration for the *GUS* knock-in *TOR/tor-1*  
585 heterozygous line (Menand *et al.*, 2002) than for a *pYAK1::YAK1-GUS* homozygous line (Barrada *et*  
586 *al.*, 2019). Therefore, the dose-dependent effect of asTORis on the processes analyzed should be  
587 preliminary determined prior to design specific genetic screen. We would like to finish this section  
588 with guidelines that might help plan experiments with TOR inhibitors in plants and algae, with an  
589 emphasis on pharmacogenetic screening.

#### 590 Guidelines for plant physiology studies and genetic screens with asTORis :

- 591 - Clarify the question you want to answer to choose the best developmental or growth stage of the  
592 plant or algae to study or to screen,

- 593 - carefully define growing conditions as growth can be widely influenced and disturbed by even  
594 subtle changes,
- 595 - choose selective drug(s) which has(ve) been best characterized, consider any possible drawback,  
596 - check the vehicle drug harmless on cell growth and keep its concentration constant whatever  
597 the drug concentration used; usually DMSO 0.1% works well in mammalian cells, plants and algae  
598 (Montane and Menand, 2013; Prioretti *et al.*, 2017; Thoreen *et al.*, 2009),
- 599 - choose clear-cut parameter (s) to quantify effect of the drug,
- 600 - establish a dose-response curve in a  $\log_{10}$  way from ca. pM including concentrations active in other  
601 species (see text and Table1),
- 602 - check stability of inhibition over time,
- 603 - check the reversibility of the drug effect if the drug is cytostatic (asTORis),
- 604 - confirm selectivity of the effect with other asTORis,
- 605 - choose a concentration related to the question you ask within the dose-response range, to avoid off  
606 target at too high doses,
- 607 - after selection of a mutant of interest, check its dose-response curve.

608 If you think you have discovered a direct target of TOR:

- 609 - check the drug-dependent effect on different organs or tissues to avoid bulk response that can mask  
610 discrete tissue responses,
- 611 - design peptide(s) encompassing the putative phosphorylation site(s) to demonstrate which one is  
612 TOR-dependent,
- 613 - express the WT and mutated form (s) in phosphorylated amino acid of the new target in a knock-  
614 out mutant and compare dose response curves.

615

616 **Conclusion**

617 If rapamycin has opened the study of TOR functions in plant and algae as in yeast and mammals, it  
618 should be used cautiously in plants for which overexpression of FKBP12 is required. The use of  
619 rapamycin has fewer drawbacks in some algae, like *Chlamydomonas reinhardtii*, which is naturally  
620 sensitive to rapamycin. However, its good potency masks incomplete efficacy in many if not all  
621 species studied till now. The requirement of FKBP12 partner to inhibit TOR with rapamycin might  
622 also disturb signaling responses due to the cellular role of FKBP12 and putative off targets of  
623 rapamycin itself might also interfere. However, in any case, we should keep in mind that rapamycin  
624 does not inhibit all TORC1 activity and does not inhibit other TOR complexes potentially present in  
625 plants and algae. Conversely, ATP-competitive TOR inhibitors more efficiently inhibit proliferation  
626 and growth than rapamycin in algae and plants, as in animals, and are therefore very good tools to  
627 study the TOR pathway in photosynthetic eukaryotes. However, we should in return take care of  
628 information's about concentration range and singularity from chemical and animal researchers who  
629 developed and experienced them. Their use has already helped decipher TOR pathway effectors in  
630 plants and algae and we guess they will certainly be of great help in the future.

631

## 632 **Supplementary data**

633 Figure S1

634

## 635 **Acknowledgements**

636 Work on TOR in our laboratory was supported by Agence Nationale de la Recherche (ANR) grants  
637 SIGNAUXBioNRJ (ANR-15-CE05-0021-03), TRANSLATOR (ANR-11-BSV6-0010) and  
638 DECORATORS (ANR-14-CE19-0007). We apologize to our colleagues whose work could not be  
639 included due to space limitations. No conflict of interest declared.

640

641

## 642 **References**

- 643 **Aagaard-Tillery K. M., Jelinek D. F.** 1994. Inhibition of Human B-Lymphocyte Cell-Cycle Progression and  
644 Differentiation by Rapamycin. *Cellular Immunology* **156**, 493-507.
- 645 **Aghdasi B., Ye K., Resnick A., Huang A., Ha H. C., Guo X., Dawson T. M., Dawson V. L., Snyder S. H.** 2001.  
646 FKBP12, the 12-kDa FK506-binding protein, is a physiologic regulator of the cell cycle. *Proceedings of the*  
647 *National Academy of Sciences, USA* **98**, 2425-2430.
- 648 **Ahn C. S., Han J. A., Lee H. S., Lee S., Pai H. S.** 2011. The PP2A regulatory subunit Tap46, a component of the  
649 TOR signaling pathway, modulates growth and metabolism in plants. *The Plant Cell* **23**, 185-209.
- 650 **Alavilli H., Lee H., Park M., Yun D. J., Lee B. H.** 2018. Enhanced multiple stress tolerance in Arabidopsis by  
651 overexpression of the polar moss peptidyl prolyl isomerase FKBP12 gene. *Plant Cell Reports* **37**, 453-465.
- 652 **Alessi D. R., Pearce L. R., Garcia-Martinez J. M.** 2009. New Insights into mTOR Signaling: mTORC2 and Beyond.  
653 *Science Signaling* **2**.
- 654 **Anderson G. H., Veit B., Hanson M. R.** 2005. The Arabidopsis AtRaptor genes are essential for post-embryonic  
655 plant growth. *BMC Biology* **3**, 12.
- 656 **Andrs M., Korabecny J., Jun D., Hodny Z., Bartek J., Kuca K.** 2015. Phosphatidylinositol 3-Kinase (PI3K) and  
657 phosphatidylinositol 3-kinase-related kinase (PIKK) inhibitors: importance of the morpholine ring. *Journal of*  
658 *Medical Chemistry* **58**, 41-71.
- 659 **Arrowsmith C. H., Audia J. E., Austin C., et al.** 2015. The promise and peril of chemical probes. *Nature*  
660 *Chemical Biology* **11**, 536-541.
- 661 **Barbet N. C., Schneider U., Helliwell S. B., Stansfield I., Tuite M. F., Hall M. N.** 1996. TOR controls translation  
662 initiation and early G1 progression in yeast. *Molecular Biology of the Cell* **7**, 25-42.
- 663 **Barrada A., Djendli M., Desnos T., Mercier R., Robaglia C., Montane M. H., Menand B.** 2019. A TOR-YAK1  
664 signaling axis controls cell cycle, meristem activity and plant growth in Arabidopsis. *Development*  
665 **doi:10.1242/dev.171298**.
- 666 **Bassham D. C.** 2009. Function and regulation of macroautophagy in plants. *Biochimica et Biophysica Acta*  
667 **1793**, 1397-1403.
- 668 **Batool A., Aashaq S., Andrabi K. I.** 2017. Reappraisal to the study of 4E-BP1 as an mTOR substrate - A  
669 normative critique. *European Journal of Cell Biology* **96**, 325-336.
- 670 **Bello T., Gujral T. S.** 2018. Kinohin: A Kinase Inhibitor Selection Portal. *iScience* **8**, 49-53.
- 671 **Ben-Sahra I., Manning B. D.** 2017. mTORC1 signaling and the metabolic control of cell growth. *Current Opinion*  
672 *in Cell Biology* **45**, 72-82.
- 673 **Benjamin D., Colombi M., Moroni C., Hall M. N.** 2011. Rapamycin passes the torch: a new generation of mTOR  
674 inhibitors. *Nature Reviews Drug Discovery* **10**, 868-880.
- 675 **Betz C., Hall M. N.** 2013. Where is mTOR and what is it doing there? *Journal of Cell Biology* **203**, 563-574.
- 676 **Blagosklonny M. V.** 2012. Cell cycle arrest is not yet senescence, which is not just cell cycle arrest: terminology  
677 for TOR-driven aging. *Aging (Albany NY)* **4**, 159-165.
- 678 **Caldana C., Li Y., Leisse A., Zhang Y., Bartholomaeus L., Fernie A. R., Willmitzer L., Giavalisco P.** 2013.  
679 Systemic analysis of inducible target of rapamycin mutants reveal a general metabolic switch controlling  
680 growth in Arabidopsis thaliana. *The Plant Journal* **73**, 897-909.
- 681 **Carlson C. B., Robers M. B., Vogel K. W., Machleidt T.** 2009. Development of LanthaScreen cellular assays for  
682 key components within the PI3K/AKT/mTOR pathway. *Journal of Biomolecular Screening* **14**, 121-132.
- 683 **Chen S. M., Liu J. L., Wang X., Liang C., Ding J., Meng L. H.** 2012. Inhibition of tumor cell growth, proliferation  
684 and migration by X-387, a novel active-site inhibitor of mTOR. *Biochemistry and Pharmacology* **83**, 1183-1194.
- 685 **Choi J., Chen J., Schreiber S. L., Clardy J.** 1996. Structure of the FKBP12-rapamycin complex interacting with  
686 the binding domain of human FRAP. *Science* **273**, 239-242.
- 687 **Chresta C. M., Davies B. R., Hickson I., et al.** 2012. AZD8055 is a potent, selective, and orally bioavailable ATP-  
688 competitive mammalian target of rapamycin kinase inhibitor with in vitro and in vivo antitumor activity.  
689 *Cancer Research* **70**, 288-298.

690 **Couso I., Evans B. S., Li J., Liu Y., Ma F., Diamond S., Allen D. K., Umen J. G.** 2016. Synergism between Inositol  
691 Polyphosphates and TOR Kinase Signaling in Nutrient Sensing, Growth Control, and Lipid Metabolism in  
692 *Chlamydomonas*. *The Plant Cell* **28**, 2026-2042.

693 **Crespo J. L., Diaz-Troya S., Florencio F. J.** 2005. Inhibition of target of rapamycin signaling by rapamycin in the  
694 unicellular green alga *Chlamydomonas reinhardtii*. *Plant Physiology* **139**, 1736-1749.

695 **Cunningham J. T., Rodgers J. T., Arlow D. H., Vazquez F., Mootha V. K., Puigserver P.** 2007. mTOR controls  
696 mitochondrial oxidative function through a YY1-PGC-1 $\alpha$  transcriptional complex. *Nature* **450**, 736-740.

697 **Davis M. I., Hunt J. P., Herrgard S., Ciceri P., Wodicka L. M., Pallares G., Hocker M., Treiber D. K., Zarrinkar P.**  
698 **P.** 2011. Comprehensive analysis of kinase inhibitor selectivity. *Nature Biotechnology* **29**, 1046-1051.

699 **De Cicco M., Rahim M. S., Dames S. A.** 2015. Regulation of the Target of Rapamycin and Other  
700 Phosphatidylinositol 3-Kinase-Related Kinases by Membrane Targeting. *Membranes (Basel)* **5**, 553-575.

701 **Deng K., Dong P., Wang W., et al.** 2017. The TOR Pathway Is Involved in Adventitious Root Formation in  
702 *Arabidopsis* and Potato. *Frontiers in Plant Science* **8**, 784.

703 **Deng K., Yu L., Zheng X., Zhang K., Wang W., Dong P., Zhang J., Ren M.** 2016. Target of Rapamycin Is a Key  
704 Player for Auxin Signaling Transduction in *Arabidopsis*. *Frontiers in Plant Science* **7**, 291.

705 **Deprost D., Truong H. N., Robaglia C., Meyer C.** 2005. An *Arabidopsis* homolog of RAPTOR/KOG1 is essential  
706 for early embryo development. *Biochemical and Biophysical Research Communications* **326**, 844-850.

707 **Dobrenel T., Caldana C., Hanson J., Robaglia C., Vincentz M., Veit B., Meyer C.** 2016a. TOR Signaling and  
708 Nutrient Sensing. *Annual Review of Plant Biology* **67**, 261-285.

709 **Dobrenel T., Mancera-Martinez E., Forzani C., et al.** 2016b. The *Arabidopsis* TOR Kinase Specifically Regulates  
710 the Expression of Nuclear Genes Coding for Plastidic Ribosomal Proteins and the Phosphorylation of the  
711 Cytosolic Ribosomal Protein S6. *Frontiers in Plant Science* **7**, 1611.

712 **Dong P., Xiong F., Que Y., Wang K., Yu L., Li Z., Ren M.** 2015. Expression profiling and functional analysis  
713 reveals that TOR is a key player in regulating photosynthesis and phytohormone signaling pathways in  
714 *Arabidopsis*. *Frontiers in Plant Science* **6**, 677.

715 **Dong Q., Mao K., Duan D., et al.** 2018. Genome-wide analyses of genes encoding FK506-binding proteins  
716 reveal their involvement in abiotic stress responses in apple. *BMC Genomics* **19**, 707.

717 **Dowling R. J., Topisirovic I., Alain T., et al.** 2010a. mTORC1-mediated cell proliferation, but not cell growth,  
718 controlled by the 4E-BPs. *Science* **328**, 1172-1176.

719 **Dowling R. J., Topisirovic I., Fonseca B. D., Sonenberg N.** 2010b. Dissecting the role of mTOR: lessons from  
720 mTOR inhibitors. *Biochimica et Biophysica Acta* **1804**, 433-439.

721 **Dunlop E. A., Tee A. R.** 2013. The kinase triad, AMPK, mTORC1 and ULK1, maintains energy and nutrient  
722 homeostasis. *Biochemical Society Transactions* **41**, 939-943.

723 **Duvel K., Yecies J. L., Menon S., et al.** 2010. Activation of a Metabolic Gene Regulatory Network Downstream  
724 of mTOR Complex 1. *Molecular Cell* **39**, 171-183.

725 **Eltschinger S., Loewith R.** 2016. TOR Complexes and the Maintenance of Cellular Homeostasis. *Trends in Cell*  
726 *Biology* **26**, 148-159.

727 **Estrada A. A., Shore D. G., Blackwood E., et al.** 2013. Pyrimidoaminotropans as potent, selective, and  
728 efficacious small molecule kinase inhibitors of the mammalian target of rapamycin (mTOR). *Journal of Medical*  
729 *Chemistry* **56**, 3090-3101.

730 **Feldman M. E., Apsel B., Uotila A., Loewith R., Knight Z. A., Ruggero D., Shokat K. M.** 2009. Active-site  
731 inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. *PLoS Biology* **7**, e38.

732 **Fraser C., Carragher N. O., Unciti-Broceta A.** 2016. ECF309: a potent, selective and cell-permeable mTOR  
733 inhibitor. *MedChemComm* **7**, 471-477.

734 **Fulcher N., Sablowski R.** 2009. Hypersensitivity to DNA damage in plant stem cell niches. *Proceedings of the*  
735 *National Academy of Sciences, USA* **106**, 20984-20988.

736 **Garcia-Echeverria C.** 2011. Blocking the mTOR pathway: a drug discovery perspective. *Biochemical Society*  
737 *Transactions* **39**, 451-455.

738 **Garcia-Martinez J. M., Moran J., Clarke R. G., Gray A., Cosulich S. C., Chresta C. M., Alessi D. R.** 2009. Ku-  
739 0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR). *Biochemical Journal* **421**, 29-42.

740 **Gaubitz C., Prouteau M., Kusmider B., Loewith R.** 2016. TORC2 Structure and Function. Trends in Biochemical  
741 Sciences **41**, 532-545.

742 **Geisler M., Bailly A.** 2007. Tete-a-tete: the function of FKBP in plant development. Trends in Plant Science **12**,  
743 465-473.

744 **Giubellino A., Bullova P., Nolting E., et al.** 2013. Combined Inhibition of mTORC1 and mTORC2 Signaling  
745 Pathways Is a Promising Therapeutic Option in Inhibiting Pheochromocytoma Tumor Growth: In Vitro and In  
746 Vivo Studies in Female Athymic Nude Mice. Endocrinology **154**, 646-655.

747 **Gold L. I., Sung J. J., Siebert J. W., Longaker M. T.** 1997. Type I (RI) and type II (RII) receptors for transforming  
748 growth factor-beta isoforms are expressed subsequent to transforming growth factor-beta ligands during  
749 excisional wound repair. American Journal of Pathology **150**, 209-222.

750 **Gonzalez S., Rallis C.** 2017. The TOR Signaling Pathway in Spatial and Temporal Control of Cell Size and  
751 Growth. Frontiers in Cell and Developmental Biology **5**, 61.

752 **Guertin D. A., Sabatini D. M.** 2007. Defining the role of mTOR in cancer. Cancer Cell **12**, 9-22.

753 **Guertin D. A., Sabatini D. M.** 2009. The pharmacology of mTOR inhibition. Science Signaling **2**, pe24.

754 **Hall M. N.** 2016. TOR and paradigm change: cell growth is controlled. Molecular Biology of the Cell **27**, 2804-  
755 2806.

756 **Hanson K. K., Ressurreicao A. S., Buchholz K., et al.** 2013. Torins are potent antimalarials that block  
757 replenishment of Plasmodium liver stage parasitophorous vacuole membrane proteins. Proceedings of the  
758 National Academy of Sciences, USA **110**, E2838-E2847.

759 **Harwood F. C., Klein Geltink R. I., O'Hara B. P., et al.** 2018. ETV7 is an essential component of a rapamycin-  
760 insensitive mTOR complex in cancer. Science Advances **4**, eaar3938.

761 **Heitman J., Movva N. R., Hall M. N.** 1991. Targets for cell cycle arrest by the immunosuppressant rapamycin in  
762 yeast. Science **253**, 905-909.

763 **Henriques R., Magyar Z., Monardes A., et al.** 2010. Arabidopsis S6 kinase mutants display chromosome  
764 instability and altered RBR1-E2F pathway activity. EMBO Journal **29**, 2979-2993.

765 **Hisanaga T., Ferjani A., Horiguchi G., et al.** 2013. The ATM-dependent DNA damage response acts as an  
766 upstream trigger for compensation in the fas1 mutation during Arabidopsis leaf development. Plant Physiology  
767 **162**, 831-841.

768 **Huang S. L., Bjornsti M. A., Houghton P. J.** 2003. Rapamycins - Mechanism of action and cellular resistance.  
769 Cancer Biology & Therapy **2**, 222-232.

770 **Huggins D. J., Sherman W., Tidor B.** 2012. Rational approaches to improving selectivity in drug design. Journal  
771 of Medicinal Chemistry **55**, 1424-1444.

772 **Imamura S., Ishiwata A., Watanabe S., Yoshikawa H., Tanaka K.** 2013. Expression of budding yeast FKBP12  
773 confers rapamycin susceptibility to the unicellular red alga Cyanidioschyzon merolae. Biochemical and  
774 Biophysical Research Communications **439**, 264-269.

775 **Imamura S., Kawase Y., Kobayashi I., Shimojima M., Ohta H., Tanaka K.** 2016. TOR (target of rapamycin) is a  
776 key regulator of triacylglycerol accumulation in microalgae. Plant Signaling & Behavior **11**, e1149285.

777 **Jhanwar-Uniyal M., Amin A. G., Cooper J. B., Das K., Schmidt M. H., Murali R.** 2017. Discrete signaling  
778 mechanisms of mTORC1 and mTORC2: Connected yet apart in cellular and molecular aspects. Advances in  
779 Biological Regulation **64**, 39-48.

780 **Juppner J., Mubeen U., Leisse A., Caldana C., Wiszniewski A., Steinhauser D., Gialalisco P.** 2018. The target  
781 of rapamycin kinase affects biomass accumulation and cell cycle progression by altering carbon/nitrogen  
782 balance in synchronized Chlamydomonas reinhardtii cells. The Plant Journal **93**, 355-376.

783 **Kamada Y., Yoshino K., Kondo C., Kawamata T., Oshiro N., Yonezawa K., Ohsumi Y.** 2010. Tor Directly  
784 Controls the Atg1 Kinase Complex To Regulate Autophagy. Molecular and Cellular Biology **30**, 1049-1058.

785 **Kang S. A., Pacold M. E., Cervantes C. L., et al.** 2013. mTORC1 phosphorylation sites encode their sensitivity to  
786 starvation and rapamycin. Science **341**, 1236566.

787 **Karaman M. W., Herrgard S., Treiber D. K., et al.** 2008. A quantitative analysis of kinase inhibitor selectivity.  
788 Nature Biotechnology **26**, 127-132.

789 **Kazyken D., Kaz Y., Kiyan V., Zhylkibayev A. A., Chen C. H., Agarwal N. K., Sarbassov dos D.** 2014. The nuclear  
790 import of ribosomal proteins is regulated by mTOR. Oncotarget **5**, 9577-9593.

791 **Kim J., Kundu M., Viollet B., Guan K. L.** 2011. AMPK and mTOR regulate autophagy through direct  
792 phosphorylation of Ulk1. *Nature Cell Biology* **13**, 132-U171.

793 **Kravchenko A., Citerne S., Jehanno I., Bersimbaev R. I., Veit B., Meyer C., Leprince A. S.** 2015. Mutations in  
794 the Arabidopsis Lst8 and Raptor genes encoding partners of the TOR complex, or inhibition of TOR activity  
795 decrease abscisic acid (ABA) synthesis. *Biochemical and Biophysical Research Communications* **467**, 992-997.

796 **Leiber R. M., John F., Verhertbruggen Y., Diet A., Knox J. P., Ringli C.** 2010. The TOR pathway modulates the  
797 structure of cell walls in Arabidopsis. *The Plant Cell* **22**, 1898-1908.

798 **Li F. Q., Vierstra R. D.** 2014. Arabidopsis ATG11, a scaffold that links the ATG1-ATG13 kinase complex to  
799 general autophagy and selective mitophagy. *Autophagy* **10**, 1466-1467.

800 **Li L., Song Y., Wang K., Dong P., Zhang X., Li F., Li Z., Ren M.** 2015. TOR-inhibitor insensitive-1 (TRIN1)  
801 regulates cotyledons greening in Arabidopsis. *Frontiers in Plant Science* **6**, 861.

802 **Li X., Cai W., Liu Y., et al.** 2017. Differential TOR activation and cell proliferation in Arabidopsis root and shoot  
803 apices. *Proceedings of the National Academy of Sciences, USA* **114**, 2765-2770.

804 **Li Y., Mitsuhashi S., Ikejo M., Miura N., Kawamura T., Hamakubo T., Ubukata M.** 2012. Relationship between  
805 ATM and ribosomal protein S6 revealed by the chemical inhibition of Ser/Thr protein phosphatase type 1.  
806 *Bioscience, Biotechnology, and Biochemistry* **76**, 486-494.

807 **Liu Q., Chang J. W., Wang J., et al.** 2010. Discovery of 1-(4-(4-propionylpiperazin-1-yl)-3-  
808 (trifluoromethyl)phenyl)-9-(quinolin-3-yl)benz o[h][1,6]naphthyridin-2(1H)-one as a highly potent, selective  
809 mammalian target of rapamycin (mTOR) inhibitor for the treatment of cancer. *Journal of Medicinal Chemistry*  
810 **53**, 7146-7155.

811 **Liu Q., Kirubakaran S., Hur W., et al.** 2012a. Kinome-wide selectivity profiling of ATP-competitive mammalian  
812 target of rapamycin (mTOR) inhibitors and characterization of their binding kinetics. *Journal of Biological*  
813 *Chemistry* **287**, 9742-9752.

814 **Liu Q., Ren T., Fresques T., et al.** 2012b. Selective ATP-competitive inhibitors of TOR suppress rapamycin-  
815 insensitive function of TORC2 in *Saccharomyces cerevisiae*. *ACS Chemical Biology* **7**, 982-987.

816 **Liu Q., Xu C., Kirubakaran S., et al.** 2013. Characterization of Torin2, an ATP-competitive inhibitor of mTOR,  
817 ATM, and ATR. *Cancer Research* **73**, 2574-2586.

818 **Liu Y. M., Bassham D. C.** 2010. TOR Is a Negative Regulator of Autophagy in Arabidopsis thaliana. *Plos One* **5**.

819 **Luo Y., Liu L., Wu Y., Singh K., Su B., Zhang N., Liu X., Shen Y., Huang S.** 2015. Rapamycin inhibits mSin1  
820 phosphorylation independently of mTORC1 and mTORC2. *Oncotarget* **6**, 4286-4298.

821 **Magnuson B., Ekim B., Fingar D. C.** 2012. Regulation and function of ribosomal protein S6 kinase (S6K) within  
822 mTOR signalling networks. *Biochemical Journal* **441**, 1-21.

823 **Mahfouz M. M., Kim S., Delauney A. J., Verma D. P.** 2006. Arabidopsis TARGET OF RAPAMYCIN interacts with  
824 RAPTOR, which regulates the activity of S6 kinase in response to osmotic stress signals. *The Plant Cell* **18**, 477-  
825 490.

826 **Marshall G., Howard Z., Dry J., et al.** 2011. Benefits of mTOR kinase targeting in oncology: pre-clinical  
827 evidence with AZD8055. *Biochemical Society Transactions* **39**, 456-459.

828 **Marshall R. S., Li F. Q., Gemperline D. C., Book A. J., Vierstra R. D.** 2015. Autophagic Degradation of the 26S  
829 Proteasome Is Mediated by the Dual ATG8/Ubiqutin Receptor RPN10 in Arabidopsis. *Molecular Cell* **58**, 1053-  
830 1066.

831 **Martelli A. M., Buontempo F., McCubrey J. A.** 2018. Drug discovery targeting the mTOR pathway. *Clinical*  
832 *Science* **132**, 543-568.

833 **Masclaux-Daubresse C., Chen Q. W., Have M.** 2017. Regulation of nutrient recycling via autophagy. *Current*  
834 *Opinion in Plant Biology* **39**, 8-17.

835 **Menand B., Desnos T., Nussaume L., Berger F., Bouchez D., Meyer C., Robaglia C.** 2002. Expression and  
836 disruption of the Arabidopsis TOR (target of rapamycin) gene. *Proceedings of the National Academy of*  
837 *Sciences, USA* **99**, 6422-6427.

838 **Meyuhas O.** 2015. Ribosomal Protein S6 Phosphorylation: Four Decades of Research. *International Review of*  
839 *Cell and Molecular Biology* **320**, 41-73.

840 **Meyuhas O., Dreazen A.** 2009. Ribosomal protein S6 kinase from TOP mRNAs to cell size. *Progress in*  
841 *Molecular Biology and Translational Science* **90**, 109-153.

842 **Michel M. C., Seifert R.** 2015. Selectivity of pharmacological tools: implications for use in cell physiology. A  
843 review in the theme: Cell signaling: proteins, pathways and mechanisms. *American Journal of Physiology-Cell*  
844 *Physiology* **308**, C505-520.

845 **Mohammed B., Biloei S. F., Doczi R., Grove E., Railo S., Palme K., Ditengou F. A., Bogre L., Lopez-Juez E.**  
846 2018. Converging Light, Energy and Hormonal Signaling Control Meristem Activity, Leaf Initiation, and Growth.  
847 *Plant Physiology* **176**, 1365-1381.

848 **Montane M. H., Menand B.** 2013. ATP-competitive mTOR kinase inhibitors delay plant growth by triggering  
849 early differentiation of meristematic cells but no developmental patterning change. *Journal of Experimental*  
850 *Botany* **64**, 4361-4374.

851 **Moreau M., Azzopardi M., Clement G., et al.** 2012. Mutations in the Arabidopsis homolog of LST8/GbetaL, a  
852 partner of the target of Rapamycin kinase, impair plant growth, flowering, and metabolic adaptation to long  
853 days. *The Plant Cell* **24**, 463-481.

854 **Mortensen D. S., Perrin-Ninkovic S. M., Shevlin G., et al.** 2015. Discovery of mammalian target of rapamycin  
855 (mTOR) kinase inhibitor CC-223. *Journal of Medicinal Chemistry* **58**, 5323-5333.

856 **Mosby's Medical Dictionary.** 2009. Retrieved October 31 2018 from [https://medical-](https://medical-dictionary.thefreedictionary.com/drug+potency)  
857 [dictionary.thefreedictionary.com/drug+potency](https://medical-dictionary.thefreedictionary.com/drug+potency).

858 **Mubeen U., Juppner J., Alpers J., Hinch D. K., Giavalisco P.** 2018. Target of Rapamycin Inhibition in  
859 *Chlamydomonas reinhardtii* Triggers de-novo Amino Acid Synthesis by Enhancing Nitrogen Assimilation. *The*  
860 *Plant Cell*.

861 **Mukaida S., Ogawa T., Ohishi K., Tanizawa Y., Ohta D., Arita M.** 2016. The effect of rapamycin on biodiesel-  
862 producing protist *Euglena gracilis*. *Bioscience Biotechnology and Biochemistry* **80**, 1223-1229.

863 **Mukhopadhyay S., Frias M. A., Chatterjee A., Yellen P., Foster D. A.** 2016. The Enigma of Rapamycin Dosage.  
864 *Molecular Cancer Therapeutics* **15**, 347-353.

865 **Nazio F., Strappazon F., Antonioli M., et al.** 2013. mTOR inhibits autophagy by controlling ULK1  
866 ubiquitylation, self-association and function through AMBRA1 and TRAF6. *Nature Cell Biology* **15**, 406-416.

867 **Nowak P., Cole D. C., Brooijmans N., et al.** 2009. Discovery of potent and selective inhibitors of the  
868 mammalian target of rapamycin (mTOR) kinase. *Journal of Medicinal Chemistry* **52**, 7081-7089.

869 **Osmulski P. A., Gaczynska M.** 2013. Rapamycin allosterically inhibits the proteasome. *Molecular*  
870 *Pharmacology* **84**, 104-113.

871 **Ouibrahim L., Rubio A. G., Moretti A., Montane M. H., Menand B., Meyer C., Robaglia C., Caranta C.** 2015.  
872 Potyviruses differ in their requirement for TOR signalling. *Journal of General Virology* **96**, 2898-2903.

873 **Pancha I., Shima H., Higashitani N., Igarashi K., Higashitani A., Tanaka K., Imamura S.** 2018. Target of  
874 rapamycin-signaling modulates starch accumulation via glycogenin phosphorylation status in the unicellular  
875 red alga *Cyanidioschyzon merolae*. *The Plant Journal*, doi.org/10.1111/tpj.14136.

876 **Park H., Choe H., Hong S.** 2014. Virtual screening and biochemical evaluation to identify new inhibitors of  
877 mammalian target of rapamycin (mTOR). *Bioorganic & Medicinal Chemistry Letters* **24**, 835-838.

878 **Patrick R. M., Lee J. C. H., Teetsel J. R. J., Yang S. H., Choy G. S., Browning K. S.** 2018. Discovery and  
879 characterization of conserved binding of eIF4E 1 (CBE1), a eukaryotic translation initiation factor 4E-binding  
880 plant protein. *Journal of Biological Chemistry* **293**, 17240-17247.

881 **Pei Z., Blackwood E., Liu L., et al.** 2012. Discovery and Biological Profiling of Potent and Selective mTOR  
882 Inhibitor GDC-0349. *ACS Medicinal Chemistry Letters* **4**, 103-107.

883 **Peng T., Golub T. R., Sabatini D. M.** 2002. The immuno suppress ant rapamycin mimics a starvation-like signal  
884 distinct from amino acid and glucose deprivation. *Molecular and Cellular Biology* **22**, 5575-5584.

885 **Perez-Perez M. E., Couso I., Crespo J. L.** 2017. The TOR Signaling Network in the Model Unicellular Green Alga  
886 *Chlamydomonas reinhardtii*. *Biomolecules* **7**.

887 **Perez-Perez M. E., Florencio F. J., Crespo J. L.** 2010. Inhibition of Target of Rapamycin Signaling and Stress  
888 Activate Autophagy in *Chlamydomonas reinhardtii*. *Plant Physiology* **152**, 1874-1888.

889 **Pfeiffer A., Janocha D., Dong Y., et al.** 2016. Integration of light and metabolic signals for stem cell activation  
890 at the shoot apical meristem. *Elife* **5**.



891 **Philippe L., Vasseur J. J., Debart F., Thoreen C. C.** 2018. La-related protein 1 (LARP1) repression of TOP mRNA  
892 translation is mediated through its cap-binding domain and controlled by an adjacent regulatory region.  
893 *Nucleic Acids Research* **46**, 1457-1469.

894 **Pike K. G., Malagu K., Hummersone M. G., et al.** 2013. Optimization of potent and selective dual mTORC1 and  
895 mTORC2 inhibitors: the discovery of AZD8055 and AZD2014. *Bioorganic & Medicinal Chemistry Letters* **23**,  
896 1212-1216.

897 **Pike K. G., Morris J., Ruston L., et al.** 2015. Discovery of AZD3147: A Potent, Selective Dual Inhibitor of  
898 mTORC1 and mTORC2. *Journal of Medicinal Chemistry* **58**, 2326-2349.

899 **Prioretti L., Avilan L., Carriere F., Montane M. H., Field B., Gregori G., Menand B., Gontero B.** 2017. The  
900 inhibition of TOR in the model diatom *Phaeodactylum tricornutum* promotes a get-fat growth regime. *Algal*  
901 *Research* **26**, 265-274.

902 **Ren M., Venglat P., Qiu S., et al.** 2012. Target of rapamycin signaling regulates metabolism, growth, and life  
903 span in *Arabidopsis*. *The Plant Cell* **24**, 4850-4874.

904 **Rexin D., Meyer C., Robaglia C., Veit B.** 2015. TOR signalling in plants. *Biochemical Journal* **470**, 1-14.

905 **Ricaud L., Proux C., Renou J. P., Pichon O., Fochesato S., Ortet P., Montane M. H.** 2007. ATM-mediated  
906 transcriptional and developmental responses to gamma-rays in *Arabidopsis*. *PLoS One* **2**, e430.

907 **Rousseau A., Bertolotti A.** 2016. An evolutionarily conserved pathway controls proteasome homeostasis.  
908 *Nature* **536**, 184-+.

909 **Roustan V., Weckwerth W.** 2018. Quantitative Phosphoproteomic and System-Level Analysis of TOR Inhibition  
910 Unravel Distinct Organellar Acclimation in *Chlamydomonas reinhardtii*. *Frontiers in Plant Science* **9**.

911 **Sabatini D. M.** 2017. Twenty-five years of mTOR: Uncovering the link from nutrients to growth. *Proceedings of*  
912 *the National Academy of Sciences, USA* **114**, 11818-11825.

913 **Salem M. A., Li Y., Bajdzienko K., Fisahn J., Watanabe M., Hoefgen R., Schottler M. A., Giavalisco P.** 2018.  
914 RAPTOR Controls Developmental Growth Transitions by Altering the Hormonal and Metabolic Balance. *Plant*  
915 *Physiology* **177**, 565-593.

916 **Sarbasov D. D., Ali S. M., Sengupta S., Sheen J. H., Hsu P. P., Bagley A. F., Markhard A. L., Sabatini D. M.**  
917 2006. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Molecular Cell* **22**, 159-168.

918 **Saxton R. A., Sabatini D. M.** 2017. mTOR Signaling in Growth, Metabolism, and Disease. *Cell* **168**, 960-976.

919 **Schepetilnikov M., Dimitrova M., Mancera-Martinez E., Geldreich A., Keller M., Ryabova L. A.** 2013. TOR and  
920 S6K1 promote translation reinitiation of uORF-containing mRNAs via phosphorylation of eIF3h. *EMBO Journal*  
921 **32**, 1087-1102.

922 **Schepetilnikov M., Kobayashi K., Geldreich A., Caranta C., Robaglia C., Keller M., Ryabova L. A.** 2011. Viral  
923 factor TAV recruits TOR/S6K1 signalling to activate reinitiation after long ORF translation. *EMBO Journal* **30**,  
924 1343-1356.

925 **Schepetilnikov M., Makarian J., Srouf O., Geldreich A., Yang Z., Chicher J., Hammann P., Ryabova L. A.** 2017.  
926 GTPase ROP2 binds and promotes activation of target of rapamycin, TOR, in response to auxin. *EMBO Journal*  
927 **36**, 886-903.

928 **Shaik A., Bhakuni R., Kirubakaran S.** 2018. Design, Synthesis, and Docking Studies of New Torin2 Analogs as  
929 Potential ATR/mTOR Kinase Inhibitors. *Molecules* **23**.

930 **Shi L., Wu Y., Sheen J.** 2018. TOR signaling in plants: conservation and innovation. *Development* **145**.

931 **Sigal N. H., Dumont F. J.** 1992. Cyclosporin A, FK-506, and rapamycin: pharmacologic probes of lymphocyte  
932 signal transduction. *Annual Review of Immunology* **10**, 519-560.

933 **Silvera D., Ernlund A., Arju R., Connolly E., Volta V., Wang J., Schneider R. J.** 2017. mTORC1 and -2 Coordinate  
934 Transcriptional and Translational Reprogramming in Resistance to DNA Damage and Replicative Stress in  
935 Breast Cancer Cells. *Molecular and Cellular Biology* **37**.

936 **Simioni C., Cani A., Martelli A. M., Zauli G., Tabellini G., McCubrey J., Capitani S., Neri L. M.** 2014. Activity of  
937 the novel mTOR inhibitor Torin-2 in B-precursor acute lymphoblastic leukemia and its therapeutic potential to  
938 prevent Akt reactivation. *Oncotarget* **5**, 10034-10047.

939 **Slotkin E. K., Patwardhan P. P., Vasudeva S. D., de Stanchina E., Tap W. D., Schwartz G. K.** 2015. MLN0128, an  
940 ATP-competitive mTOR kinase inhibitor with potent in vitro and in vivo antitumor activity, as potential therapy  
941 for bone and soft-tissue sarcoma. *Molecular Cancer Therapeutics* **14**, 395-406.

942 **Smithson L. J., Gutmann D. H.** 2016. Proteomic analysis reveals GIT1 as a novel mTOR complex component  
943 critical for mediating astrocyte survival. *Genes and Development* **30**, 1383-1388.

944 **Song Y., Zhao G., Zhang X., et al.** 2017. The crosstalk between Target of Rapamycin (TOR) and Jasmonic Acid  
945 (JA) signaling existing in Arabidopsis and cotton. *Scientific Reports* **7**, 45830.

946 **Sormani R., Yao L., Menand B., Ennar N., Lecampion C., Meyer C., Robaglia C.** 2007. *Saccharomyces*  
947 *cerevisiae* FKBP12 binds Arabidopsis thaliana TOR and its expression in plants leads to rapamycin  
948 susceptibility. *BMC Plant Biology* **7**, 26.

949 **Soto-Burgos J., Bassham D. C.** 2017. SnRK1 activates autophagy via the TOR signaling pathway in Arabidopsis  
950 thaliana. *Plos One* **12**.

951 **Sparks C. A., Guertin D. A.** 2010. Targeting mTOR: prospects for mTOR complex 2 inhibitors in cancer therapy.  
952 *Oncogene* **29**, 3733-3744.

953 **Stan R., McLaughlin M. M., Cafferkey R., Johnson R. K., Rosenberg M., Livi G. P.** 1994. Interaction between  
954 Fkbp12-Rapamycin and Tor Involves a Conserved Serine Residue. *Journal of Biological Chemistry* **269**, 32027-  
955 32030.

956 **Sturgill T. W., Hall M. N.** 2009. Activating mutations in TOR are in similar structures as oncogenic mutations in  
957 PI3K $\alpha$ . *ACS Chemical Biology* **4**, 999-1015.

958 **Suttangkakul A., Li F., Chung T., Vierstra R. D.** 2011. The ATG1/ATG13 protein kinase complex is both a  
959 regulator and a target of autophagic recycling in Arabidopsis. *The Plant Cell* **23**, 3761-3779.

960 **Tavares M. R., Pavan I. C., Amaral C. L., Meneguello L., Luchessi A. D., Simabuco F. M.** 2015. The S6K protein  
961 family in health and disease. *Life Sciences* **131**, 1-10.

962 **Thoreen C. C.** 2017. The molecular basis of mTORC1-regulated translation. *Biochemical Society Transactions*  
963 **45**, 213-221.

964 **Thoreen C. C., Kang S. A., Chang J. W., et al.** 2009. An ATP-competitive mammalian target of rapamycin  
965 inhibitor reveals rapamycin-resistant functions of mTORC1. *Journal of Biological Chemistry* **284**, 8023-8032.

966 **Thoreen C. C., Sabatini D. M.** 2009. Rapamycin inhibits mTORC1, but not completely. *Autophagy* **5**, 725-726.

967 **van Dam T. J. P., Zwartkruis F. J. T., Bos J. L., Snel B.** 2011. Evolution of the TOR Pathway. *Journal of Molecular*  
968 *Evolution* **73**, 209-220.

969 **Velazquez A. F. C., Jackson W. T.** 2018. So Many Roads: the Multifaceted Regulation of Autophagy Induction.  
970 *Molecular and Cellular Biology* **38**.

971 **Vespa L., Vachon G., Berger F., Perazza D., Faure J. D., Herzog M.** 2004. The immunophilin-interacting protein  
972 AtFIP37 from Arabidopsis is essential for plant development and is involved in trichome endoreduplication.  
973 *Plant Physiology* **134**, 1283-1292.

974 **Vezina C., Kudelski A., Sehgal S. N.** 1975. Rapamycin (Ay-22,989), a New Antifungal Antibiotic .1. Taxonomy of  
975 Producing Streptomycete and Isolation of Active Principle. *Journal of Antibiotics* **28**, 721-726.

976 **Walters H. E., Cox L. S.** 2018. mTORC Inhibitors as Broad-Spectrum Therapeutics for Age-Related Diseases.  
977 *International Journal of Molecular Science* **19**.

978 **Wang P., Mugume Y., Bassham D. C.** 2018a. New advances in autophagy in plants: Regulation, selectivity and  
979 function. *Seminars in Cell & Developmental Biology* **80**, 113-122.

980 **Wang P., Zhao Y., Li Z., et al.** 2018b. Reciprocal Regulation of the TOR Kinase and ABA Receptor Balances Plant  
981 Growth and Stress Response. *Molecular Cell* **69**, 100-112 e106.

982 **Wang R., Sunchu B., Perez V. I.** 2017. Rapamycin and the inhibition of the secretory phenotype. *Experimental*  
983 *Gerontology* **94**, 89-92.

984 **Werth E. G., McConnell E. W., Couso Lianez I., Perrine Z., Crespo J. L., Umen J. G., Hicks L. M.** 2018.  
985 Investigating the effect of target of rapamycin kinase inhibition on the Chlamydomonas reinhardtii  
986 phosphoproteome: from known homologs to new targets. *New Phytologist*.

987 **Wicker L. S., Boltz R. C., Jr., Matt V., Nichols E. A., Peterson L. B., Sigal N. H.** 1990. Suppression of B cell  
988 activation by cyclosporin A, FK506 and rapamycin. *European Journal of Immunology* **20**, 2277-2283.

989 **Xiong F., Dong P., Liu M., et al.** 2016. Tomato FK506 Binding Protein 12KD (FKBP12) Mediates the Interaction  
990 between Rapamycin and Target of Rapamycin (TOR). *Frontiers in Plant Science* **7**, 1746.

991 **Xiong F., Zhang R., Meng Z., et al.** 2017a. Brassinosteroid Insensitive 2 (BIN2) acts as a downstream effector of  
992 the Target of Rapamycin (TOR) signaling pathway to regulate photoautotrophic growth in Arabidopsis. *New*  
993 *Phytologist* **213**, 233-249.

994 **Xiong M. N., Zhu Z. P., Tian S. W., et al.** 2017b. Conditional ablation of Raptor in the male germline causes  
995 infertility due to meiotic arrest and impaired inactivation of sex chromosomes. *FASEB Journal* **31**, 3934-3949.

996 **Xiong Y., McCormack M., Li L., Hall Q., Xiang C., Sheen J.** 2013. Glucose-TOR signalling reprograms the  
997 transcriptome and activates meristems. *Nature* **496**, 181-186.

998 **Xiong Y., Sheen J.** 2012. Rapamycin and glucose-target of rapamycin (TOR) protein signaling in plants. *Journal*  
999 *of Biological Chemistry* **287**, 2836-2842.

1000 **Xu Q., Liang S. P., Kudla J., Luan S.** 1998. Molecular characterization of a plant FKBP12 that does not mediate  
1001 action of FK506 and rapamycin. *The Plant Journal* **15**, 511-519.

1002 **Yoo Y. J., Kim H., Park S. R., Yoon Y. J.** 2017. An overview of rapamycin: from discovery to future perspectives.  
1003 *Journal of Industrial Microbiology & Biotechnology* **44**, 537-553.

1004 **Yu K., Shi C., Toral-Barza L., et al.** 2010. Beyond rapalog therapy: preclinical pharmacology and antitumor  
1005 activity of WYE-125132, an ATP-competitive and specific inhibitor of mTORC1 and mTORC2. *Cancer Research*  
1006 **70**, 621-631.

1007 **Yu K., Toral-Barza L., Shi C., et al.** 2009. Biochemical, cellular, and in vivo activity of novel ATP-competitive and  
1008 selective inhibitors of the mammalian target of rapamycin. *Cancer Research* **69**, 6232-6240.

1009 **Zhang R., Meng Z., Zhou T., Deng Y., Feng L., Wang Y., Sun G., Guo S., Ren M.** 2013. ScFKBP12 bridges  
1010 rapamycin and AtTOR in Arabidopsis. *Plant Signaling & Behavior* **8**, e26115.

1011 **Zhao J., Zhai B., Gygi S. P., Goldberg A. L.** 2015. mTOR inhibition activates overall protein degradation by the  
1012 ubiquitin proteasome system as well as by autophagy. *Proceedings of the National Academy of Sciences, USA*  
1013 **112**, 15790-15797.

1014 **Zhao J. H., Garcia G. A., Goldberg A. L.** 2016. Control of proteasomal proteolysis by mTOR. *Nature* **529**, E1-E2.

1015 **Zheng Y., Jiang Y.** 2015. mTOR Inhibitors at a Glance. *Molecular and cellular pharmacology* **7**, 15-20.

1016

1017

1018

1019

1020 **Tables**

1021 **Table 1. Concentrations of rapamycin and different asTORis inhibiting TOR kinase activity**  
 1022 **(IC50), mammalian cells proliferation (IC50), WT plant root and leaf growth, and WT green**  
 1023 **alga and diatom proliferation.** Values were obtained with different *in vitro* kinase assays as well as  
 1024 different proliferation assays described in the references. Cell lines are embryonic or cancerous. Range  
 1025 of values was from different mammalian cell lines in the same article. Concentration unit is nM. Note  
 1026 that rapamycin never fully inhibits non-cancerous mammalian cell proliferation, growth of roots and  
 1027 leaves of *A. thaliana* seedlings or of the unicellular green alga *C. reinhardtii*.

Inhibitor / original paper on mammalian cells	IC50 or EC50 in <i>in vitro</i> TOR kinase	IC50 mammalian cell proliferation (MEFs <sup>a</sup> / cancerous cells <sup>b</sup> )	IC50 <i>A. thaliana</i> root lengthening <sup>c/d</sup>	Estimated doses for <i>A. thaliana</i> leaf size reduction <sup>e</sup>	Estimated IC50 <i>C. reinhardtii</i> <sup>f</sup>	IC50 <i>P. tricornutum</i> <sup>g</sup>
Rapamycin		10- 500 / <1- 20,000	No effect up to 10,000 <sup>c/d</sup> , impaired solubility beyond that <sup>c</sup>	nd	Couple of doses 100-500	Single dose, slight effect at 10,000
PP242 (Feldman <i>et al.</i> , 2009)	8	1000	nd	nd	nd	nd
Torin1 (Thoreen <i>et al.</i> , 2009)	1-10	10-250	>1,000 impaired solubility beyond that	nd	No dose response, used at 500	nd
KU-0063794 (Garcia-Martinez <i>et al.</i> , 2009)	10	1,200	5 - 6,000	nd	nd	nd
WYE-354 (Yu <i>et al.</i> , 2009)	5	200 - 2,000	2,000	nd	nd	nd
Torin2 (Liu <i>et al.</i> , 2013)	0.25-10	13 - 200	500	nd	nd	nd

AZD-8055 (Chresta <i>et al.</i> , 2012)	2.5	50	500	20µl of 7,500 to 30,000 nM per 1 cm-wide leaf and shoot apex	No dose response, routinely used at 500	4000-6000
WYE-125132 (WYE-132)  (Yu <i>et al.</i> , 2010)	0.19	24-145	200	nd	nd	< 5,000

1028 <sup>a</sup> (Thoreen *et al.*, 2009); <sup>b</sup> (Guertin and Sabatini, 2007; Huang *et al.*, 2003; Mukhopadhyay *et al.*,  
1029 2016); <sup>c</sup> (Montane and Menand, 2013); <sup>d</sup> (Ren *et al.*, 2012), note that growth IC50 of lines  
1030 overexpressing yeast FKBP12 is observed with ca. 500-1,000 nM rapamycin, <sup>e</sup> Leaves of 3 weeks old  
1031 plants grown on soil and under long days (16h) were rubbed with drops of AZD-8055 and grown for 6  
1032 days before scoring growth inhibition (Ouibrahim *et al.*, 2015). <sup>f</sup> (Crespo *et al.*, 2005; Juppner *et al.*,  
1033 2018; Roustan and Weckwerth, 2018); <sup>g</sup> (Prioretti *et al.*, 2017).

1034

1035

1036

1037

1038

1039

## 1040 **Figures legends**

1041 **Figure 1: Efficacy of rapamycin and the asTORi AZD-8055 on growth and proliferation of**  
1042 **mammalian cells, plants and algae.** Dose response curves of rapamycin (**A, C, E**) and of AZD-8055  
1043 (**B, D, F**) on mouse cancerous cells (**A-B**), Arabidopsis root growth (**C-D**) and *C. reinhardtii* growth  
1044 (**E-F**). Note that mouse embryonic fibroblasts (non-cancerous) are also partially inhibited by  
1045 rapamycin but completely by Torin1 (Thoreen *et al.*, 2009). Plant dose response to rapamycin is  
1046 shown for Wild Type (WT) and plant overexpressing *A. thaliana* (At)-, Human (Hs)-, or yeast (BP12)-  
1047 FKBP12 (**C**). For *A. thaliana*, dose response with AZD-8055 is shown for the WT and the *TOR/tor-1*

1048 heterozygote, which has higher sensitivity to this asTORi due to haploinsufficiency of *TOR*. In all  
1049 species, the efficacy of growth inhibition by rapamycin is never maximal (ca. 50%) while it is  
1050 maximal by AZD-8055. From (Giubellino *et al.*, 2013) (**A, B**), (Deng *et al.*, 2016) (**C**), (Montane and  
1051 Menand, 2013) (**D**), (Juppner *et al.*, 2018) (**E**) and (Imamura *et al.*, 2016) (**F**). Figures and images are  
1052 reproduced with permission of Oxford Academic (**A, B**), Wiley Online Library (**E**) and Taylor &  
1053 Francis (**F**) or were originally published under the Creative Commons Attribution License (**C, D**).

1054

1055

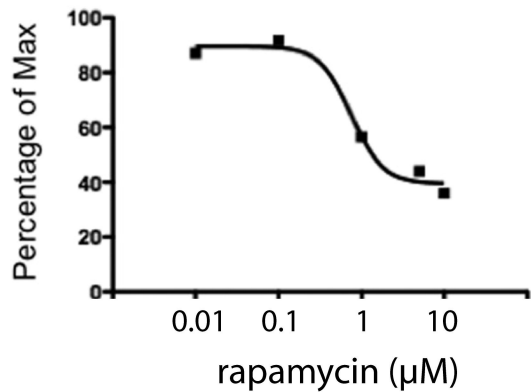
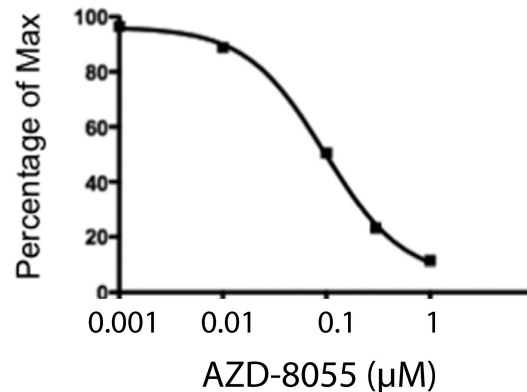
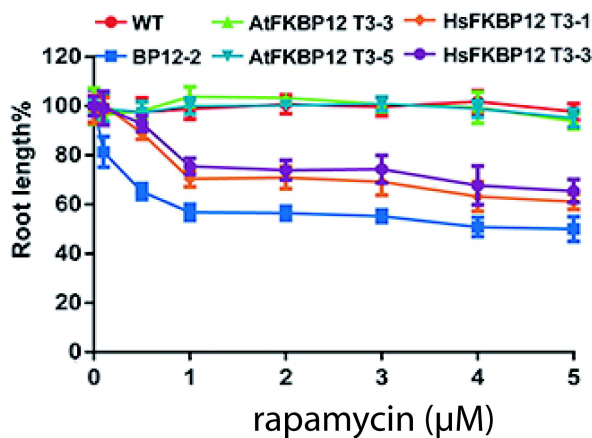
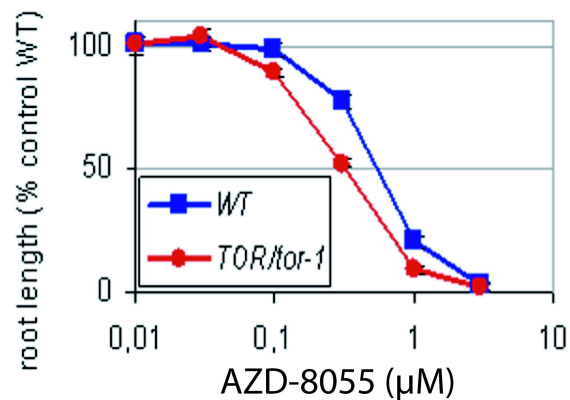
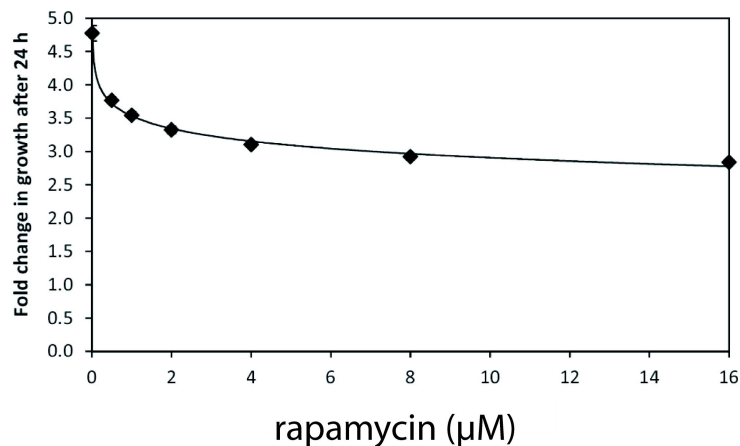
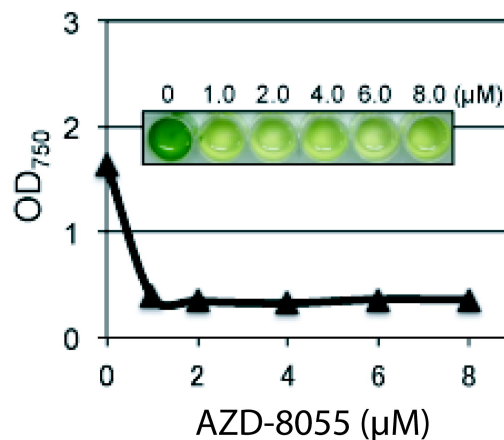
1056 **Figure 2: Formula of main asTORis discussed in this review.**

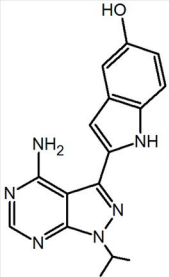
1057 For original publication of each inhibitor, see text and Table 1. Drawing was done with the  
1058 ACD/ChemSketch freeware.

1059

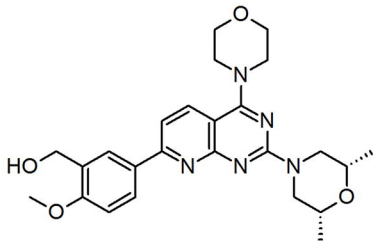
1060 **Figure 3: The quality of mTORC1 substrates determines their sensitivity to rapamycin, asTORis**  
1061 **and nutrients availability.** Poor mTORC1 substrates like S6K1 T389, 4EBP1 S65 and also GRB10  
1062 S476 are inhibited by rapamycin and partial amino acid depletion. In the other hand, strong mTORC1  
1063 targets, including ULK1 S758, 4E-BP1-T37/46, but also GRB10 S150 and PRAS40 S183, are resistant  
1064 to rapamycin but not to asTORis or complete starvation. From data of (Kang *et al.*, 2013).

1065

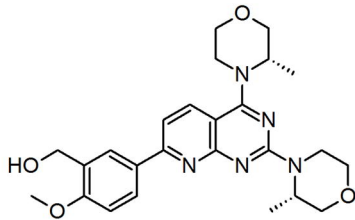
**A** metastatic mouse pheochromocytoma-derived cell line**B** metastatic mouse pheochromocytoma-derived cell line**C** *Arabidopsis thaliana***D** *Arabidopsis thaliana***E** *Chlamydomonas reinhardtii***F** *Chlamydomonas reinhardtii*



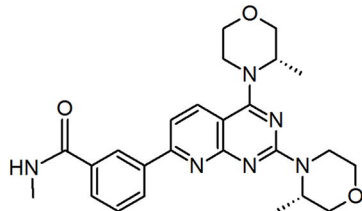
PP242



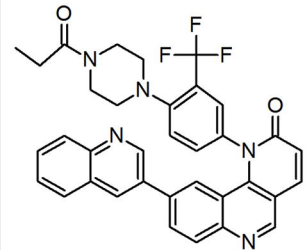
KU-0063794



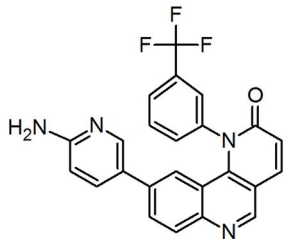
AZD-8055



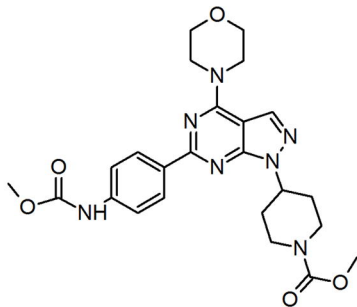
AZD-2014



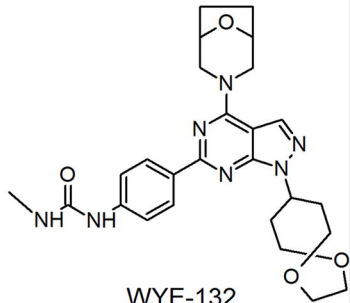
Torin1



Torin2



WYE-354

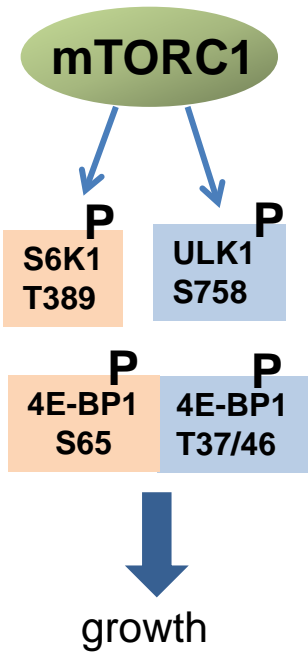


WYE-132



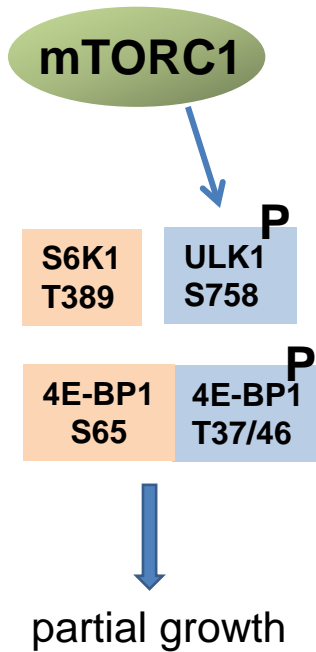
### fully active

100% aminoacids



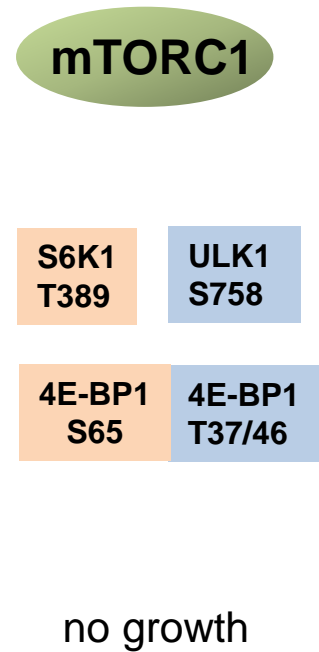
### partial inhibition

20% aminoacids  
or rapamycin



### strong inhibition

0% aminoacids  
or asTORis



 strong mTORC1 targets

 poor mTORC1 targets