

1 Cell cycle control by the Target of Rapamycin signalling pathway in 2 plants

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10

11 Abstract

12

13 Cells need to ensure a sufficient nutrient and energy supply before committing to proliferate.
14 In response to positive mitogenic signals, such as light, sugar availability and hormones, the
15 TARGET OF RAPAMYCIN (TOR) signalling pathway promotes cell growth that connects to
16 the entry and passage through the cell division cycle via multiple signalling mechanisms.
17 Here, we summarise current understanding of cell cycle regulation by the RBR-E2F
18 regulatory hub and the DREAM-like complexes, and highlight possible functional relations
19 between these regulators and TOR signalling. A genetic screen recently uncovered a
20 downstream signalling component to TOR that regulates cell proliferation, YAK1, a member
21 of the dual specificity tyrosine phosphorylation regulated kinase (DYRK) family. YAK1
22 activates the plant-specific SIAMESE-RELATED (SMR) cyclin-dependent kinase inhibitors
23 and therefore could be important to regulate both CDKA-RBR-E2F pathway to control the
24 G1/S and the mitotic CDKB1;1 to control the G2/M transitions. TOR, as a master regulator of
25 both protein synthesis-driven cell growth and cell proliferation is also central for cell size
26 homeostasis. We conclude the review by briefly highlighting the potential applications of
27 combining TOR and cell cycle knowledge in context of ensuring future food security.

28

29 Introduction

30 In plants, the cell cycle activity is concentrated in pools of undifferentiated cells, called
31 meristems and this activity is the major driver for above- and below-ground organ growth
32 (Gazquez and Beemster, 2017). Being energetically expensive, cell production, however, is
33 limited by sugar availability and is dependent on sugar-sensing signalling pathways centred
34 around the antagonistically acting Target of Rapamycin (TOR) and Sucrose Non-fermenting-
35 related kinase 1 (SnRK1; Dobrenel *et al.*, 2016; Lastdrager *et al.*, 2014; Rexin *et al.*, 2015).
36 In this review, we will discuss our current understanding on how light and sucrose regulates
37 meristem activities through modulating the cell cycle. Because of the functional and
38 structural conservation of both TOR pathway components and core cell cycle regulators, we
39 will also highlight relevant yeast and animal literature to make a case for possible plant TOR
40 and cell cycle connections.

41 TOR was discovered in budding yeast through the block of cell cycle progression in the G1
42 phase of the cell cycle upon treatment with rapamycin, a bacterial compound specifically
43 targeting TOR. However, unlike mutants in genes controlling the cell cycle that continue to
44 grow without cell division to become large, the rapamycin-treated yeast cells were small,
45 leading to the original idea that TOR is a principal regulator of cell growth and through this
46 indirectly effects cell cycle progression (Wang and Proud, 2009). Therefore, it is surprising
47 that in plants TOR can directly regulate the expression of cell cycle genes and thus cell
48 proliferation (Xiong *et al.*, 2013). However, there is accumulating evidence that TOR as in
49 other organisms, also regulates translation and through this meristem activity and cell
50 proliferation (Schepetilnikov and Ryabova, 2018).

51 It is well accepted that growth drives cell cycle in many different organisms and being tightly
52 connected to maintain cell size homeostasis (Amodeo and Skotheim, 2016; Wood and
53 Nurse, 2015). The involvement of TOR in this process is evident in yeast, animal cells and
54 might also be the case for plant meristematic cells, but the exact mechanism is not yet
55 known (Sablowski and Carnier Dornelas, 2014). TOR is commonly considered to control the
56 G1/S transition of the cell cycle but there is evidence specifically in the context of cell size
57 homeostasis that it also acts through the G2/M control (Wood and Nurse, 2015). We will
58 review the information available on sucrose and light control of the plant cell cycle to see
59 how distinct cell cycle control points might be utilised. For general reviews on how plant
60 relevant external conditions impact on plant physiology through the TOR signalling pathway,
61 readers are referred to other excellent reviews (Dobrenel *et al.*, 2016; Lastdrager *et al.*,
62 2014; Rexin *et al.*, 2015; Shi *et al.*, 2018).

63

64 **TOR signalling promotes cell proliferation both in shoot and root meristems**

65 The *Arabidopsis TOR-promoter::GUS* transcriptional reporter is highly expressed in the
66 primary meristem, but not in differentiated cells, indicating that TOR function is largely
67 restricted to the meristematic region (Barrada *et al.*, 2019; Menand *et al.*, 2002). Both in
68 TOR silenced plants and plants treated with TOR-specific ATP-competitive inhibitors e.g.
69 AZD8055, there is a clear reduction in root and shoot growth. The dose-dependent inhibition
70 of root growth by TOR inhibitors was traced back to the reduction of meristem size (Barrada
71 *et al.*, 2019; Montane and Menand, 2013; Xiong *et al.*, 2013). This was done by measuring
72 cell size profiles to determine the point where cells exit the cell cycle and start to elongate in
73 the root meristem, by visualising mitotic cells using pCYCB1;1::destruction box-GUS reporter
74 or by visualising cells in S-phase by EdU labelling. Thus, TOR regulates how long cells
75 maintain the proliferation competence in the meristem before exiting to cell elongation and
76 differentiation.

77 Both shoot and root growth are reliant on photosynthates and TOR-dependent activation of
78 cell proliferation (Mohammed *et al.*, 2018; Pfeiffer *et al.*, 2016; Wu *et al.*, 2019; Xiong *et al.*,
79 2013). In the shoot, to maintain meristem activity, it was suggested that in addition to sugar,
80 auxin biosynthesis is also required that is stimulated by blue and red light receptors and the
81 COP1 signalosome to activate the TOR kinase Fig1A; (Chen *et al.*, 2018; Li *et al.*, 2017).
82 The light, sugar and hormonal requirement for the activation of shoot meristem was also
83 examined during the developmental transition of deetiolation (Chen *et al.*, 2018; Mohammed
84 *et al.*, 2018). The dark-arrested meristem is under a state of energy deprivation
85 accompanied by diffused auxin and non-membrane PIN1 localisation (Mohammed *et al.*,

86 2018). The non-polar PIN1 localisation is instigated at least partly by the MKK7-MPK6
87 mitogen activated signalling module and the direct phosphorylation of PIN1 by MPK6 (Dóczy
88 *et al.*, 2019; Dory *et al.*, 2018). Upon light exposure there is a rapid release of the starvation
89 response, PIN1 expression is induced by light (Lopez-Juez *et al.*, 2008) and becomes polar
90 to remove auxin towards the growing leaf primordia (Dóczy *et al.*, 2019; Mohammed *et al.*,
91 2018). This is followed by the COP1 light signalling dependent induction of cell cycle- and
92 protein translation-associated genes. For cell cycle regulation COP1 alters the balance
93 between the activator E2FB and the repressor E2FC transcription factors (Berckmans *et al.*,
94 2011; Lopez-Juez *et al.*, 2008). The rapid and transient decline in the expression of auxin
95 responsive genes e.g *AUX1* upon light exposure is not dependent on the
96 photomorphogenesis program (Mohammed *et al.*, 2018). Light requirement for leaf
97 emergence can be bypassed in the dark by altering the auxin-cytokinin signalling balance,
98 for example lowering the auxin response in the *axr1*, or increasing the cytokinin response in
99 the *arr1* mutants or by the exogenous supply of cytokinin or sucrose to the dark arrested
100 shoot primordia (Braybrook and Kuhlemeier, 2010; Mohammed *et al.*, 2018; Yoshida *et al.*,
101 2011). This TOR-dependent sugar signal alone in the dark is perfectly capable to stimulate
102 cell proliferation, but the development of a normal leaf lamina requires photomorphogenesis-
103 like hormonal responses (Mohammed *et al.*, 2018).

104 It was shown that auxin signalling is relayed to TOR through Rho-related protein 2 (ROP2; a
105 member of the Rho GTPase family; Li *et al.*, 2017; Schepetilnikov *et al.*, 2017). TOR
106 activation promotes cell cycle entry by activating E2FA and E2FB transcription factors (Li
107 *et al.*, 2017). The auxin induced ROP2-TOR pathway also plays important role in gene-specific
108 translational control (Schepetilnikov *et al.*, 2017; Schepetilnikov and Ryabova, 2017). The
109 translationally controlled root and shoot meristem development and cell cycle target mRNAs
110 by TOR are not yet established. In a physiological setting, TOR signalling has an important
111 role to tune the extent of cell cycle activity and growth of young leaves non-cell
112 autonomously under varying light irradiance (Mohammed *et al.*, 2018).

113 Light and TOR signalling also regulate cell proliferation in single-cell plants such as the green
114 alga *Chlamydomonas* (Perez-Perez *et al.*, 2017). The *Chlamydomonas* proliferates through
115 a multiple-fission mechanism in which a long growth phase can precede multiple DNA
116 replication rounds followed by multiple numbers of division, thereby producing two, four or
117 eight daughter cells. The number of divisions normally depends on the light intensity and
118 consequently the mother cell size (Bisova and Zachleder, 2014; Umen, 2018). The allosteric
119 TOR inhibitor rapamycin suppressed division of *Chlamydomonas*, but increased the cell size
120 at both early (within 1h) and later time-points (20h and 24h) after the treatment. Moreover,
121 rapamycin delayed the onset of commitment point and mitosis, but interestingly not S phase
122 progression (Juppner *et al.*, 2018). These results suggest that in *Chlamydomonas* TOR acts
123 on important cell cycle regulatory transitions both in G1/S and G2/M, as well as it regulates
124 cell size. The principal regulator of the commitment point is the RBR gene; *MAT3* in
125 *Chlamydomonas*. CDKG1 was identified as an RBR kinase in this organism that determines
126 the number of mitosis and consequent cell size in relation to mother cell size dictated by light
127 (Li *et al.*, 2016b; Umen, 2018). Based on the cell cycle outcomes of TOR inhibition, the
128 CDKG1-MAT3 module represent a plausible signalling target for TOR to regulate these cell
129 cycle transitions (Fig 2).

130

131 **Control of G1/S progression by the TOR pathway**

132 A conserved hallmark of commitment to enter the cell cycle is centred on the inactivation of a
133 nuclear G1/S repressor, the Retinoblastoma protein (Rb), in plants called RB-RELATED
134 (RBR). The inactivation occurs through phosphorylation by CDKA-CYCD complexes on
135 multiple conserved residues of RBR, which results in the release of E2F-type transcription
136 factors from RBR binding and allows for the transcription of genes required for DNA
137 replication (Magyar *et al.*, 2016).

138 In *Arabidopsis*, there is a single RBR-coding gene, and the *rbr1* null-mutant alleles show
139 gametophytic lethality, because the megagametophyte fails to arrest mitosis and undergoes
140 excessive nuclear proliferation in the embryo sac (Ebel *et al.*, 2004). Silencing of *RBR* with
141 RNA interference leads to continued proliferation and the lack of cellular differentiation in
142 developing leaves (Borghi *et al.*, 2010). Likewise, co-suppression of *RBR* (csRBR) due to the
143 introduction of an extra copy, resulted in a complete growth arrest of *Arabidopsis* seedlings
144 in nutrient limited conditions. At the same time, cells in developing cotyledons of csRBR
145 seedlings showed gross over-proliferation when sucrose was supplemented in the growth
146 medium (Gutzat *et al.*, 2011). This raised the possibility for the existence of an unknown
147 growth repressor independent or below RBR, which leads to the halt of cell proliferation in
148 nutrient limited conditions.

149 Downstream of RBR, there are three E2F transcription factors (E2FA, E2FB and E2FC),
150 which associate with one of the DIMERISATION PARTNER proteins (DPA or DPB) for DNA
151 binding (Magyar, 2008). Mainly on the basis of overexpression studies, E2Fs can be
152 categorised as activators (E2FA and B) or repressor-type (E2FC; Harashima and Sugimoto,
153 2016). In response to growth stimulating conditions, such as plant hormones or the available
154 sugars, the abundance of particular G1 cyclin increases (Riou-Khamlichi *et al.*, 2000).
155 CYCD-CDKA;1 complexes then hyper-phosphorylate RBR on multiple conserved sites that
156 leads to the release of activator E2Fs from RBR-binding to induce the expression of
157 cell-cycle genes (Magyar *et al.*, 2012; Nakagami *et al.*, 2002). In contrast, the repressor-type
158 E2Fs function together with RBR to block cell proliferation. It is emerging that the separation
159 into these two categories are sometimes blurred. For instance, the two E2Fs with positive
160 roles in cell proliferation; E2FA and E2FB exhibit clear functional differences. When cell
161 proliferation was induced by either applying exogenous sucrose or elevating CYCD3;1
162 levels, the complex formation between E2FB and RBR was disrupted due to RBR
163 phosphorylation, however the interactions between E2FA and RBR were not weakened, but
164 even further enhanced (Magyar *et al.*, 2012). Based on ectopic expression studies, RBR-free
165 E2FB regulates both G1/S and G2/M transition, and represses endoreduplication (Magyar *et al.*
166 *et al.*, 2005; Sozzani *et al.*, 2006). A recent *in vivo* phosphoproteomics analysis upon TOR
167 inhibition uncovered that RBR phosphorylation on the CDKA sites are regulated by TOR
168 activity. At the same time, E2Fs were not found to be TOR-dependently phosphorylated in
169 this phosphoproteomics screen (Van Leene *et al.*, 2019). In another recent study, it was
170 shown that TOR inhibits the expression of SIAMESE-RELATED (SMR) cyclin-dependent
171 kinase inhibitors through the YAK1 kinase (Fig1A; Barrada *et al.*, 2019). Whether the TOR-
172 dependent RBR phosphorylation by CDKA activity relies on changing cyclin or the opposing
173 CDK inhibitor (CKI) abundance remains to be investigated.

174 The RBR-E2FA complex was shown to have a role in repressing endocycle genes (Fig1A),
175 such as *CCS52A1* and *CCS52A2* in the meristem, thus preventing premature exit of cells to

176 the elongation zone and therefore maintaining a healthy pool of dividing cells (Magyar *et al.*,
177 2012). It might be feasible that TOR phosphorylation on E2FA promotes the formation of
178 such a repressor RBR-E2FA complex to increase meristem size and therefore organ growth
179 in the presence of sucrose. It might also be possible that TOR only phosphorylates RBR-free
180 E2FA, which promotes S-phase progression during mitotic cell cycle and endocycle when
181 cells elongate (Xiong *et al.*, 2013).

182 In response to glucose induction, TOR makes global transcriptome changes, including many
183 S-phase regulatory genes (Xiong *et al.*, 2013). It was shown that in *Arabidopsis* cells TOR is
184 able to interact with E2FA and when immuno-precipitated from seedlings, TOR could *in vitro*
185 phosphorylate the recombinant E2FA within a large region of its N-terminus (1-80 amino
186 acid), but the exact phosphorylation sites have not yet been determined (Xiong *et al.*, 2013).
187 Because a broad-spectrum S/T protein kinase inhibitor, staurosporine did not affect the
188 TOR-dependent E2FA activation, it was also concluded that S6K is not required downstream
189 of TOR for the activation of S-phase genes (Xiong *et al.*, 2013). After deleting the 80aa N-
190 terminal region, E2FA lost its transcriptional activity, but it is not clear whether such
191 truncated E2FA retains its ability for DNA binding. In a similar experimental setup, TOR was
192 also shown to phosphorylate E2FB (Li *et al.*, 2017), even though the N-terminal domains and
193 specifically the distribution of phosphorylation sites on E2FA and E2FB greatly differ from
194 each other. It was further shown that TOR, E2FA and E2FB are all important to activate the
195 root meristem of *Arabidopsis* plants from an experimentally-induced oxygen-deprived
196 quiescent state. Based on the direct interaction and phosphorylation of E2FA and E2FB by
197 TOR, it was proposed that the TOR-E2FA/B regulatory unit is independent of the canonical
198 CDK-CYC-RBR route of cell cycle entry. It will be of importance to determine the exact
199 phosphorylation sites on these E2F proteins and how these phosphorylation events regulate
200 their functions in terms of DNA binding, transactivation of target genes, association with RBR
201 and other regulatory proteins.

202 The *Arabidopsis* mutant line, where the neighbouring S6K1 and S6K2 genes were both
203 deleted by a T-DNA insertion and rearrangement, shows sterility and aneuploidy (Henriques
204 *et al.*, 2010). This suggested a role for S6K in meiosis and chromosome segregation during
205 male and female gametogenesis and in somatic cells. Investigating the mechanism behind
206 this mitotic defect led to the discovery that S6K1 interacts with RBR and E2FB proteins, and
207 required for the nuclear localisation of RBR (Henriques *et al.*, 2010). To find out the
208 physiological relevance for this molecular interaction, S6K1 was silenced in cultured cells
209 grown with or without sucrose. While cell division was completely inhibited without sucrose,
210 the S6K1-silenced cells continued to divide, showing that under nutrient starvation
211 conditions, S6K1 functions as a repressor of cell proliferation (Henriques *et al.*, 2010).
212 Further supporting the repressor function of S6K1 in cell division that it downregulates E2FB
213 protein level, while E2FB negatively regulates S6K protein level and activity (Henriques *et al.*,
214 2013). Such double negative loops are characteristic of molecular switches, this
215 particular S6K1-RBR-E2FB circuit could serve to repress cell proliferation upon energy
216 exhaustion, which can be reversed to induce cell proliferation upon sucrose availability,
217 when the TOR-S6K pathway is activated (Fig 1B; Henriques *et al.*, 2014).

218

219 **Control of G2/M progression by the TOR pathway**

220 The TOR signalling pathway is most often discussed as a regulator for G1/S transition,
221 however studies on other organisms suggest that TORC1 components also have function at
222 the onset of mitosis (Fig 2; Atkin *et al.*, 2014). In fission yeast there are two TOR proteins;
223 Tor1 and Tor2, which form two distinct complexes TORC2 and TORC1, respectively. The
224 Tor1-centred pathway is facilitating the cell growth under nutrient-limited conditions,
225 meanwhile the Tor2 signalling is responsible for vegetative growth by controlling the G1/S
226 transition. The nutrient dependent mitotic entry is mediated through Tor1 signalling and the
227 stress response MAP kinase pathway involving Sty1, leading to changes in the activity of the
228 mitotic kinase Cdc2 (Petersen and Nurse, 2007). In budding yeast, either treating cells with
229 rapamycin or introducing a temperature-sensitive allele of raptor (a conserved regulatory
230 partner of TOR), resulted in mitotic delay with a prolonged G2 phase (Nakashima *et al.*,
231 2008). In synchronised human cell lines, it was shown that raptor is mitotically
232 phosphorylated on multiple phospho-sites and required for normal G2/M transition, since
233 ectopic expression of phospho-mutant raptor caused G2/M delay (Ramirez-Valle *et al.*,
234 2010). Interestingly, the mitotic CDK1-cyclinB complex was shown to be responsible for the
235 phosphorylation of RAPTOR during M-phase in yeast (Gwinn *et al.*, 2010).

236 In plants, our understanding of TOR signalling in M-phase control is yet to be cemented. The
237 recent finding that TOR regulates cell cycle progression through the SMR class of CDK
238 inhibitor proteins hints that this might have both G1/S and G2/M inputs (Fig 2; Barrada *et al.*,
239 2019), because the SMRs were shown to act both on CDKA;1 with RBR as a major target
240 and the mitosis-specific CDKB1;1 (Kumar *et al.*, 2015). There is also evidence to suggest
241 that sucrose, a prevalent inducer of TOR, regulates the cell cycle differently at the G1/S and
242 G2/M transitions. Silencing of RBR allows sucrose-deprived *Arabidopsis* cultured cells to
243 enter into the cell cycle, but interestingly these RBR silenced cells were arrested later in the
244 cell cycle at G2 to M phase transition (Hirano *et al.*, 2008). This suggests that the
245 downregulation of RBR can bypass the starvation-induced G1-, but not the G2-arrest.
246 Similar observation was reported by Borghi *et al.* (2010) with RBR silenced (RBRi)
247 *Arabidopsis* plants, where they showed increased number of cells with 4C DNA content in
248 the leaf, suggesting a G2 arrest. Moreover, overexpression of CYCD3;1 in cell culture that
249 leads to RBR inactivation also have an increased G2 cell cycle profile (Menges *et al.*, 2006).
250 These data collectively show that RBR acts on the G1/S transition to repress the cell cycle
251 under sucrose-limiting conditions. What is the repression mechanism imposed by sucrose
252 starvation at the G2/M phase is not yet known. It might also be possible that RBR have
253 some non-canonical role at the G2/M progression to regulate chromatin structure,
254 chromosome segregation or DNA repair (Dick *et al.*, 2018; Horvath *et al.*, 2017). On the
255 mechanism of sucrose starvation-induced G2 arrest there are some clues coming from
256 developmental regulators of shoot meristem activity. Skylar and colleagues reported that
257 exogenous sucrose could revert the low activity of mitotic *CYCB1;1::GUS* and
258 *CDKB1;1::GUS* reporters in the *stip* mutant (an allele of WUSCHEL-related homeobox 9;
259 WOX9). Furthermore, sucrose induction rapidly repressed *TPR-DOMAIN SUPPRESSOR*
260 *OF STIMPY* (*TSS*) transcription to rescue the *stip* mutant G2-arrested phenotype,
261 suggesting that WOX9 regulates G2/M transition by suppressing *TSS* (Riou-Khamlichi *et al.*,
262 2000). In another study, WOX9 was shown to interact with CYCP2;1, a cyclin that physically
263 associates with three mitotic CDKs, and is required for the G2/M transition during meristem
264 activation (Peng *et al.*, 2014). Plants relay sugar availability largely through TOR pathway,
265 thus it is possible that the WOX9-G2/M axis is functionally associated with TOR activation.

266 Expression of G1/S and G2/M phase specific genes are coordinated by the E2F and the B-
267 myb transcription factors, respectively (Magyar *et al.*, 2016). Importantly, both these classes
268 of transcription factors are together part of the multiprotein complex known as DP, RB-like
269 E2F, and MuvB (DREAM) discovered in *Drosophila* and were later found in worm (DRM) and
270 mammals. The DREAMs are repressor complexes containing multiple transcription factors
271 besides E2Fs and Mybs (Sadasivam and DeCaprio, 2013).

272 Recently, DREAM-like complexes have been described in *Arabidopsis* (Fig 3; Kobayashi *et al.*,
273 2015a, Kobayashi *et al.*, 2015b, Magyar *et al.*, 2016). Specific to plants is the existence
274 of at least two distinct DREAM complexes, one with activator type transcription factors
275 (E2FB and MYB3R4) and another with repressor types (E2FC and MYB3R3, Kobayashi *et al.*,
276 2015a; Kobayashi *et al.*, 2015b; Magyar *et al.*, 2016). The activator complex can turn into
277 repressor when cells exit cell-cycle, in this situation, E2FC and MYB3R3 respectively replace
278 E2FB and MYB3R4 to inhibit expression of G2/M genes, establish quiescence and to
279 achieve a differentiation state. Another function of the repressor DREAM complex in plants
280 to repress mitotic genes outside of M-phase to ensure the waves of transcriptional activation
281 in M-phase (Kobayashi *et al.*, 2015b). In mammals, the assembly of the repressor DREAM
282 complex is regulated by the dual specificity tyrosine-phosphorylation-regulated kinase 1A
283 (DYRK1A; Guiley *et al.*, 2015). DYRK1A phosphorylates a subunit of MuvB, called LIN52,
284 which is conserved among animals but have not yet been reported in plants. This
285 phosphorylation event will serve as a signal to the DREAM complex to promote down-
286 regulation of cell cycle genes. Whether such regulation is operational in plants, and if it is
287 involved in DREAM complex assembly or the interchange between activator and repressor
288 type DREAM complexes on target genes, remains to be established.

289 Acceleration of cell cycle poses a threat of frequent of DNA damage, and to prevent passage
290 of damaged genome to the next generations, cell cycle must be halted (Maya-Mendoza *et al.*,
291 2018). Recovery from G2/M DNA damage checkpoint has been shown to dependent on
292 TORC1 in human cells (Hsieh *et al.*, 2018). TOR transcriptionally controls two of the most
293 important mitotic genes, cyclin B1 and polo-like kinase 1 (PLK1) through regulation of
294 histone lysine demethylase 4B (KDM4B). In *Arabidopsis* the upregulation of SMR-type CDK
295 inhibitors and the stabilisation of repressor-type R1R2R3-Myb transcription factors were
296 shown to suppress G2/M-specific genes to inhibit cell division in response to DNA damage
297 (Chen *et al.*, 2017). In addition, the RBR-E2FA complex was shown to localise on damaged
298 heterochromatin foci and together they act as transcriptional repressor of the orthologue of
299 the human breast cancer susceptibility gene 1 (Horvath *et al.*, 2017). Biologically, it makes
300 sense that RBR, being a master cell cycle regulator, also has a role in safeguarding the
301 genome and thus ensuring genome integrity during proliferation. Whether the DNA damage
302 response in plants is under TOR control is an open question.

303 **YAK1 emerged as a principal downstream target of TOR to regulate cell proliferation**

304 The DYRK family protein kinases are regarded as important regulators of cell cycle activity in
305 yeast and animal cells (Becker, 2012; Soppa and Becker, 2015). For instance, DYRK2
306 negatively regulates S-phase entry, since depletion of its activity accelerated S-phase
307 progression in human cells (Taira *et al.*, 2012). Another DYRK family member is YAK1,
308 which was actually the first member to be discovered through a genetic screen in search for
309 negative growth regulators in *Saccharomyces cerevisiae* (Garrett and Broach, 1989). Initially
310 in *Arabidopsis* YAK1 was reported to act as a positive mediator of abscisic acid (ABA)

311 signalling in response to drought stress (Kim *et al.*, 2015). ABA represses the expression of
312 G1/S-phase genes like *CDKA*, *CDC10 Target1 (CDT1A)*, *TOPOISOMERASE I*; and
313 promotes the expression of CDK inhibitors such as *KIP-RELATED PROTEIN 1 (KRP1)*,
314 therefore ABA signalling negatively regulates the cell cycle (Gutierrez, 2009). There is a
315 direct connection between TOR and ABA pathways, as it was shown that TOR inhibits ABA
316 signalling by phosphorylating the ABA receptor *PYRABACTIN RESISTANCE 1-like 1*
317 (*PYL1*). On the other hand, ABA represses TOR signalling by SnRK2-mediated
318 phosphorylation of *RAPTOR1* Fig 1A; (Wang *et al.*, 2018). Further, since a DYRK family
319 member is known to regulate the DREAM complex repressive function, it is tempting to
320 speculate whether TOR-regulated YAK1 signalling plays a role in modulating the activator-
321 or repressor-type DREAM complex (Fig 3).

322 Recently a genetic screen for insensitivity to TOR inhibition provided compelling evidence for
323 YAK1 to be a principal regulator below TOR to regulate root growth and meristem
324 maintenance (Barrada *et al.*, 2019). Loss-of-function YAK1 mutants are resistant to AZD-
325 8055 while YAK1 overexpressors are hypersensitive. YAK1 is essential for TOR-dependent
326 transcriptional regulation of the SMR cyclin-dependent kinase inhibitors to restrict cell
327 proliferation in the meristem. There is a possibility that YAK1 may act on TOR signalling
328 through ABA as well as downstream of TOR to regulate cell cycle progression. Recently, a
329 TOR phosphoproteomics study also uncovered YAK1 as a possible TOR target to be
330 phosphorylated on at least two phosphopeptides (Van Leene *et al.*, 2019).

331

332 **TOR-dependent translational control of the progression through the cell cycle**

333 Control at the translational level allows faster accumulation of the necessary cell cycle
334 components compared with the regulation of transcription. The connection between the
335 TOR-regulated translation initiation and cell cycle progression was first uncovered in budding
336 yeast, where TOR was shown to be required for the eIF-4E-dependent protein synthesis
337 and, thereby, G1 progression in response to nutrient availability by enhanced translation of a
338 G1 cyclin, *CLN3* (Fig 2; Barbet *et al.*, 1996). TOR also controls the proliferation of animal
339 cells through selective translation of cell cycle regulatory genes, including cyclin D3 (Fig 2;
340 Dowling *et al.*, 2010). In agreement to these yeast and animal literature, a study using
341 *Arabidopsis* cell culture showed that sucrose starvation induces the translational repression
342 of genes enriched in cell cycle and cell growth (Nicolai *et al.*, 2006). Diurnal regulation of
343 translation also has large impact on the translational regulation of mRNAs including cell
344 cycle regulators (Missra *et al.*, 2015). Photomorphogenesis is another example
345 accompanied by global changes in translationally controlled mRNA recruitment to polysomes
346 (Liu *et al.*, 2012; Liu *et al.*, 2013). De-etiolating *Arabidopsis* seedlings undergo a rapid
347 increase in translational capacity through phyA mediated repression of *COP1*, which acts
348 negatively on auxin signalling. Upon *COP1* inhibition, auxin-activated TOR induces the
349 phosphorylation of the Ribosomal Protein S6 (*RPS6*) and it was suggested that this acts as
350 a trigger for translation (Chen *et al.*, 2018). However, the role of *RPS6* phosphorylation by
351 TOR-mediated *S6K* activation on translation is debated in yeast and animal literature,
352 because mutating the phosphorylation sites on *RPS6* has no effect on protein translation
353 (Ruvinsky and Meyuhas, 2006; Yerlikaya *et al.*, 2016). Interesting, *RPS6* also have functions
354 outside the ribosome as it was shown to associate with plant-specific histone deacetylase
355 *HD2* family members on rRNA gene promoters to regulate ribosome biogenesis (Kim *et al.*,

356 2014). In animal cells Rb also have a role to regulate ribosome biogenesis through
357 transcriptional repression of PolII and PolIII promoters (White, 2005).

358 Other components of the mRNA translation machinery have also been implicated in cell
359 cycle regulation. The eIF3h protein is part of the translation initiation complex, regulates the
360 selective translation of mRNAs containing upstream open reading frames in their 5' UTR.
361 eIF3h activity is regulated by the TOR signalling through S6K1-mediated phosphorylation
362 (Schepetilnikov *et al.*, 2013). The *eif3h* mutant showed enhanced expression of *WUSCHEL*
363 and *CLAVATA3* in the apical shoot meristem, leading to over-proliferation and enlarged
364 meristematic region, suggesting that eif3h provide a translational control in meristem
365 maintenance (Zhou *et al.*, 2014).

366 The ErbB-3 epidermal growth factor receptor binding protein (EBP1) is an evolutionary
367 conserved growth regulator (Stegmann, 2018). In the plant field EBP1 came into the
368 limelight as a dose dependent regulator of organ growth that in meristematic cells promote
369 cell proliferation while in post mitotic cells it enhances cellular growth (Horvath *et al.*, 2006).
370 EBP1 was also identified as a potential gene involved in hybrid vigour. EBP1 expression is
371 largely concentrated to the plant meristems and it was shown to be regulated by TOR
372 (Deprost *et al.*, 2007). Moreover, EBP1 expression shows strong co-regulation with a large
373 group of genes having gene annotation of translational control, suggesting that EBP1 might
374 enhance plant growth through this mechanism (Horvath *et al.*, 2006). In animal cells EBP1 is
375 localised to the nucleus, the nucleolus and the cytoplasm. In the nucleolus of human cells,
376 EBP1, as part of ribonucleoprotein complexes, interacts with different rRNA species,
377 therefore presumably plays a role in ribosome biogenesis (Squatrino *et al.*, 2004). In the
378 cytosol, EBP1 is associated with mature ribosomes and inhibits the stress-induced
379 phosphorylation of the eukaryotic initiation factor 2 alpha (eIF2a), therefore positively
380 regulating the mRNA translation (Squatrino *et al.*, 2006). In the nucleus, EBP1 physically
381 binds to E2F1, Rb, histone deacetylase 2 (HDAC2) and Sin3A, therefore contributes to
382 transcriptional repression of E2F targets and other growth regulator genes (Zhang *et al.*,
383 2005). In contrast to animal cells, in plant cells EBP1 was shown to have a positive effect on
384 cell proliferation and to positively regulate the expression of E2F target genes. In part, this
385 might be through the downregulation of RBR protein level by EBP1.

386 Taken together, EBP1 and eIF3h studies show the relevance of translation-dependent
387 control of cell cycle progression in plants. The TOR-EBP1-RBR, TOR-S6K-S6 and the TOR-
388 S6K-eIF3h interactions are perhaps involved in matching and tuning cell growth with cell
389 cycle progression both at the levels of translation initiation and ribosome biogenesis.

390

391 **Maintaining cell size homeostasis whilst cycling, the TOR connection**

392 Although cell growth (increase in size) and cell division (increase in cell number) are two
393 separate processes with distinct regulation, but they are tightly coupled to maintain cell size
394 homeostasis (Amodeo and Skotheim, 2016; Sablowski and Carnier Dornelas, 2014; Umen,
395 2018). TOR is the master regulator of protein synthesis (a driver of cell growth), but coupled
396 to cell cycle regulation by multiple mechanisms. In fission yeast, deletion of Tor1 results in
397 mildly larger cells under nutrient-rich growth conditions suggesting that TOR limits the onset
398 of mitosis through MAPK signalling to allow more time for cell growth to occur and thus,
399 increasing final cell size at division (Fig 2; Petersen and Nurse, 2007). In mammalian cell

400 culture systems, blocking TOR using rapamycin leads to smaller cells regulated at both G1/S
401 and G2/M points, but the effect is more pronounced at the former transition point (Fingar *et al.*,
402 2004). The molecular basis of cell size regulation in cycling cells by TOR involves its
403 well-conserved effector S6K1 activity 4E-BP1/eukaryotic translation initiation factor 4E
404 (Fingar *et al.*, 2004).

405 In *Arabidopsis*, overexpression of G1/S cyclin CYCD3;1 results in reduced cell size (Dewitte
406 *et al.*, 2003; Jones *et al.*, 2017) phenocopied when E2FB expression is elevated in tobacco
407 BY-2 cells (Magyar *et al.*, 2005). In the *Arabidopsis* shoot meristem, mathematical modelling
408 coupled with time-course microscopy work, it was reported that transition into both S-phase
409 and M-phase is size-dependent (Jones *et al.*, 2017), which is in agreement with the yeast
410 studies. Additionally, increasing or decreasing CDK production, respectively, leads to smaller
411 and larger meristematic cells. Thus, CDK activity drives size-dependent progression through
412 the cell cycle. Considering that (i) RBR phosphorylation is the principal target of CDKA
413 activity (ii) E2FB overexpression and RBR silencing results in reduced cell size, and (iii)
414 E2FB is involved in the regulation of both G1/S and G2/M transition, the TOR-YAK1-SMR-
415 CDKA-RBR-E2FB axis should be important to couple cell growth and cell cycle progression
416 in the context of organ size control and cell size homeostasis. This might explain why E2FB,
417 and not E2FA, can drive expression of both G1/S and G2/M genes and speed up cell cycle
418 progression (Magyar *et al.*, 2005).

419

420 **From TOR and cell cycle research to increasing crop yield**

421 Improving crop yield requires the understanding of molecular interactions and signalling
422 pathways underlying plant growth and development. Overexpression of TOR results in
423 bigger *Arabidopsis* plants (Deprost *et al.*, 2007). Similarly, overexpression of one of the TOR
424 target, EBP1 leads to increased organ growth both in *Arabidopsis*, potato and becomes
425 upregulated by hybrid vigour (Li *et al.*, 2016a). More recently, Bakshi and colleagues
426 ectopically expressed *Arabidopsis TOR* in rice and found that it increased growth and yield
427 under water-limiting conditions (Bakshi *et al.*, 2017). Furthermore, these transgenic rice lines
428 showed insensitivity to ABA at the level of seed germination (Bakshi *et al.*, 2017; Bakshi
429 *et al.*, 2019). Manipulating sugar signalling itself has also been reported to enhance crop yield.
430 For instance, chemically spraying precursors of Trehalose-6-Phosphate (T6P) in *Arabidopsis*
431 and wheat leads to increase yield and drought tolerance (Griffiths *et al.*, 2016). T6P is
432 thought to act as a signal for sucrose content (Wingler, 2018). Important future avenue is to
433 effectively transfer the knowledge we gathered on TOR signalling to address important
434 questions, such as identification of yield determining and yield stability factors connected to
435 TOR in crop plants (Bakshi *et al.*, 2019).

436

437 **Figure 1. Swirls of TOR pathways leading to cell cycle control**

438 A. Cell cycle and cell growth are continuously adjusted to environmental signals (shown in
439 red) such as sugar and light availability. Accordingly, TOR signalling cascade (shown in
440 green) regulates the cell cycle through various signalling routes (shown in blue) and cell
441 cycle regulators (shown in lilac). Light activates TOR by triggering phytochrome; phyA to
442 inhibit the E3 ligase COP1, which negatively influences auxin-ROP2 signalling to TOR. The
443 presence of sugars activate TOR, which results in the phosphorylation of E2F cell cycle

444 transcription factors. TOR is also known to positively influence the transcription of *EBP1*, a
445 regulator of cell and organ growth. At the protein level, EBP1 negatively regulates the cell
446 cycle repressor RBR, and vice versa. EBP1 promotes CYCDs transcription, thus cell cycle
447 entry. RBR in complex with E2FA represses transcription of endocycle genes in the
448 meristem. S6K1 is the most widely known effector of TOR, and it may be involved in
449 promoting translation of core cell cycle regulators such as CYCDs as in other model
450 systems. ABA signalling promotes SnRK activity, the “yang” of TOR pathway. TOR
451 counteracts ABA response through phosphorylation of its receptor PYLs. This may result in
452 promotion of cell cycle through counteracting the ABA-induced expression of CDK inhibitors
453 (CKIs). YAK1 recently emerged as a principal downstream target of TOR to regulate cell
454 cycle through the SMR type CDK inhibitors and as a regulator of ABA signalling.

455 B. The S6K1-RBR-E2FB module of the TOR network has a cell cycle repression function
456 under sucrose starvation. Nutrient deprivation inactivates TOR signalling and S6K1. In its
457 inactive state S6K1 promotes the nuclear localisation of RBR where it inhibits E2FB. S6K1
458 and E2FB negatively affect each other's protein stability. Thus, S6K1 also serves as a
459 negative regulator of cell cycle.

460

461 **Figure 2. TOR – cell cycle regulation across the kingdoms**

462 TOR is a universal master regulator of cell growth in eukaryotes that connects to cell cycle
463 regulation in various ways in different organisms. In fission yeast the nutrient dependent
464 mitotic entry is mediated through Tor1 signalling and the stress response MAP kinase
465 pathway involving Sty1, leading to changes in the activity of the mitotic kinase Cdc2 and
466 mitotic entry. Upon nutrient starvation Gad8, an AGC kinase, is activated by Tor1 signalling
467 to promote the arrest of mitotic cell cycle in G1 phase therefore cells enter sexual
468 development. In budding yeast, TOR regulates G1/S through promoting translation of G1
469 cyclin CLN3 and through de-stabilising SIC, a repressor of the CDK CDC28. TOR is also
470 shown to regulate G2/M transition by promoting the nucleocytoplasmic translocation CDC5,
471 a polo-like kinase. In mammalian cell lines, mTOR regulates translation of cell cycle
472 regulators such as CYCD through its effector S6K1. TOR signalling is also required during
473 mitosis since RAPTOR is mitotically phosphorylated by CDK1-CYCB complex. In
474 *Chlamydomonas*, G1/S and G2/M transitions are controlled by E2F-DP association and
475 CDKG1-CYCD dependent phosphorylation of RBR. Based on widespread cell cycle
476 regulation by TOR signalling, this is likely to be under TOR control. In *Arabidopsis*, TOR
477 exerts its G1/S control through directly phosphorylating E2FA and allowing transcription of
478 genes required for DNA replication. Recently, YAK1 was shown to be under TOR control.
479 YAK1 negatively regulates cell cycle through CDK family of inhibitors, the SMRs.

480 **Figure 2. TOR to DREAM**

481 The multi-protein DREAM complex transcriptionally regulates progression and repression of
482 cell cycle. Based on animal models, DRKY kinase regulate the DREAM complex assembly.
483 Recently, a member of the DRKY kinase family, the *Arabidopsis* YAK1 was shown to be
484 downstream of TOR, and a YAK1 phosphopeptide was found to be a target of TOR
485 phosphorylation. This raises the possibility that YAK1 below TOR may regulate the
486 behaviour of activator- and repressor-type DREAM complexes in a nutrient-dependent
487 manner.

488

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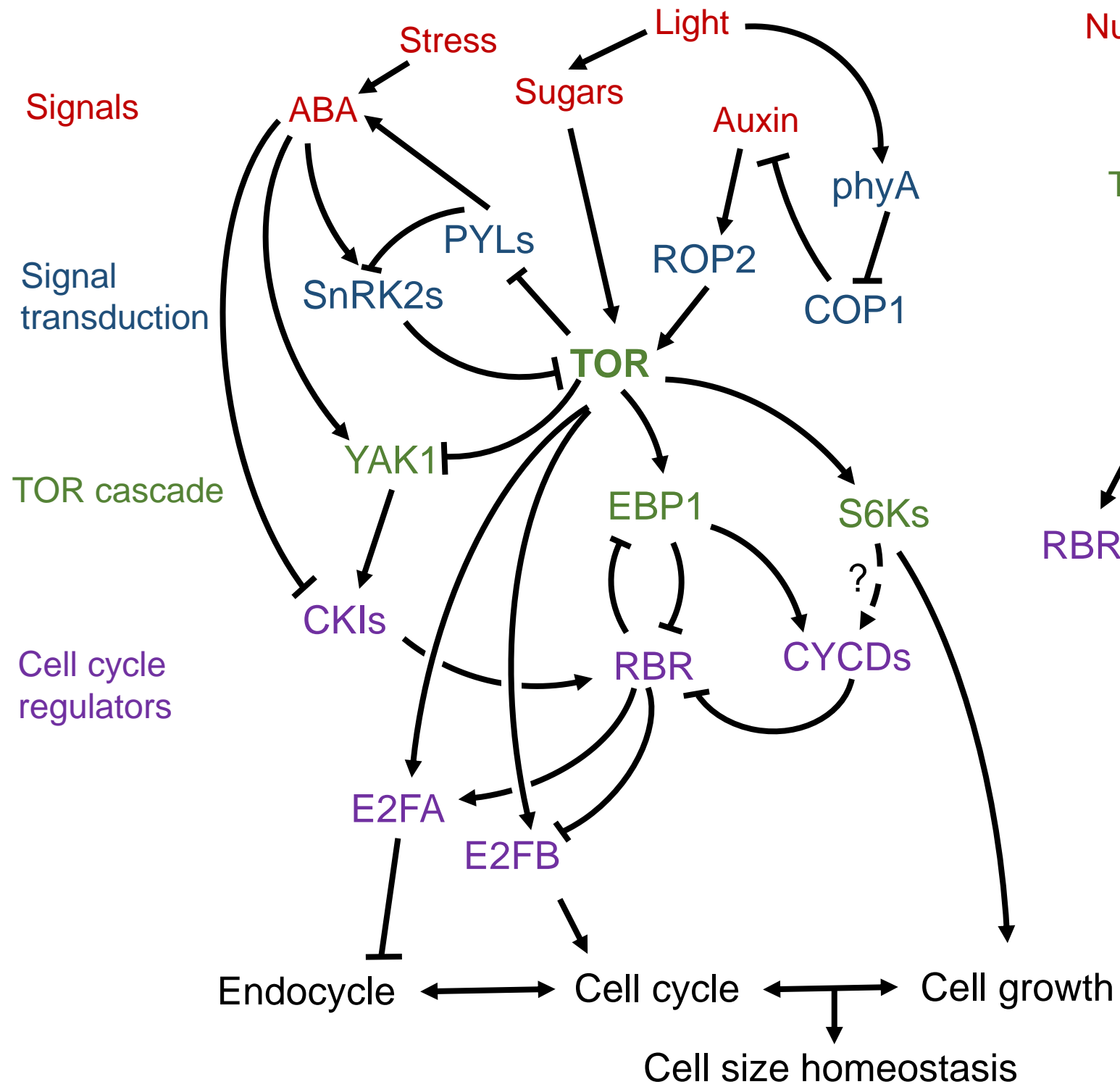
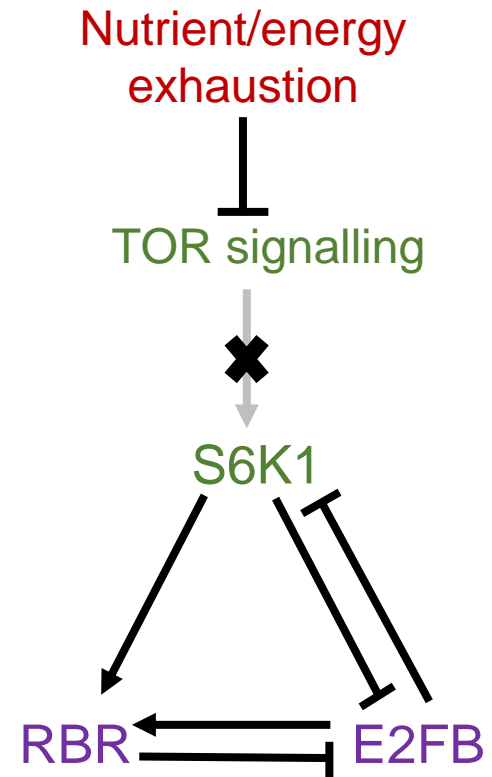
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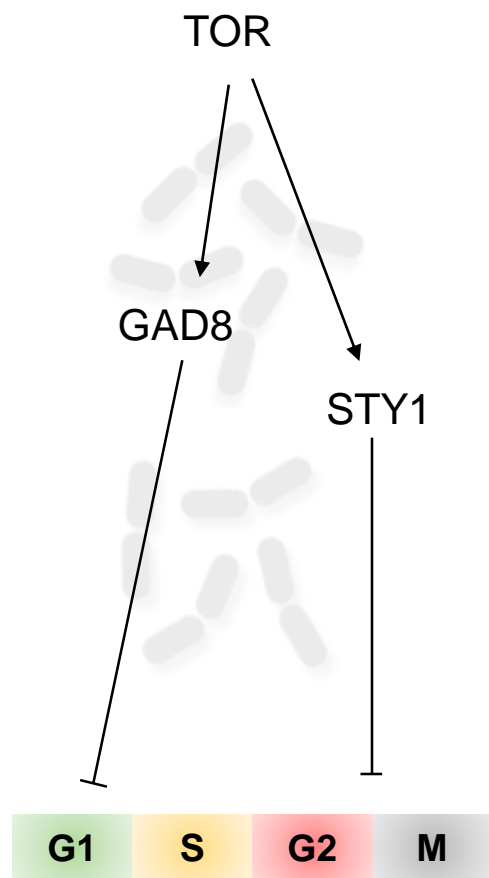
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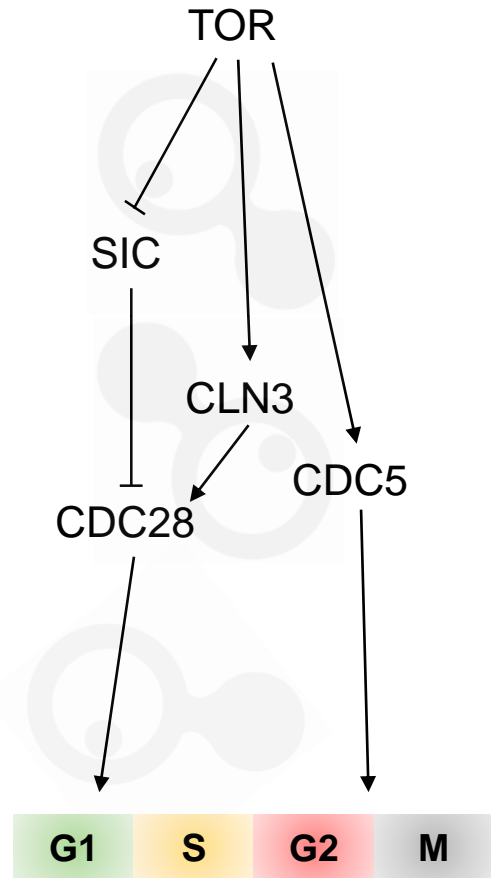
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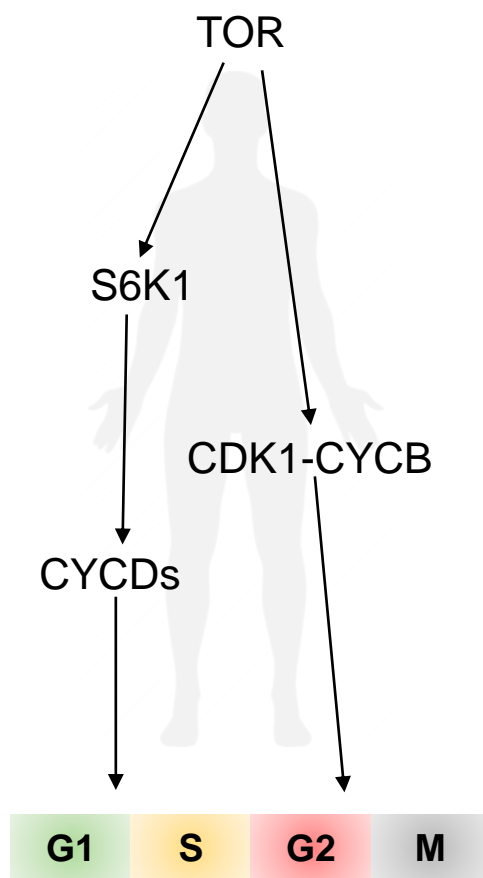
Fission yeast



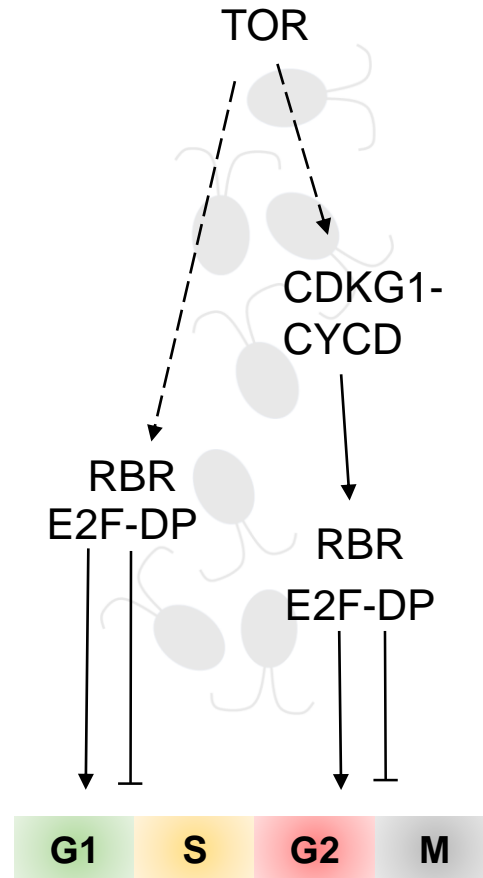
Budding yeast



Mammals



Chlamydomonas



Arabidopsis

