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

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Abstract

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

Introduction

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

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

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

TOR signalling promotes cell proliferation both in shoot and root meristems

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

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

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

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

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

Control of G1/S progression by the TOR pathway

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

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

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

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

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

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

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

Control of G2/M progression by the TOR pathway

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Maintaining cell size homeostasis whilst cycling, the TOR connection

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

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

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

From TOR and cell cycle research to increasing crop yield

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

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

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

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

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

Figure 2. TOR – cell cycle regulation across the kingdoms

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

Figure 2. TOR to DREAM

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

Acknowledgments

Z.A. is a recipient of BBSRC-DTP studentship (BB/M011178/1). L.B. and C.P. were funded by BBSRC-NSF grant BB/M025047/1. Z.M. was supported by the Hungarian Scientific Research Fund (OTKA NN-107838) and by the Ministry for National Economy (Hungary, GINOP-2.3.2-15-2016-00001).

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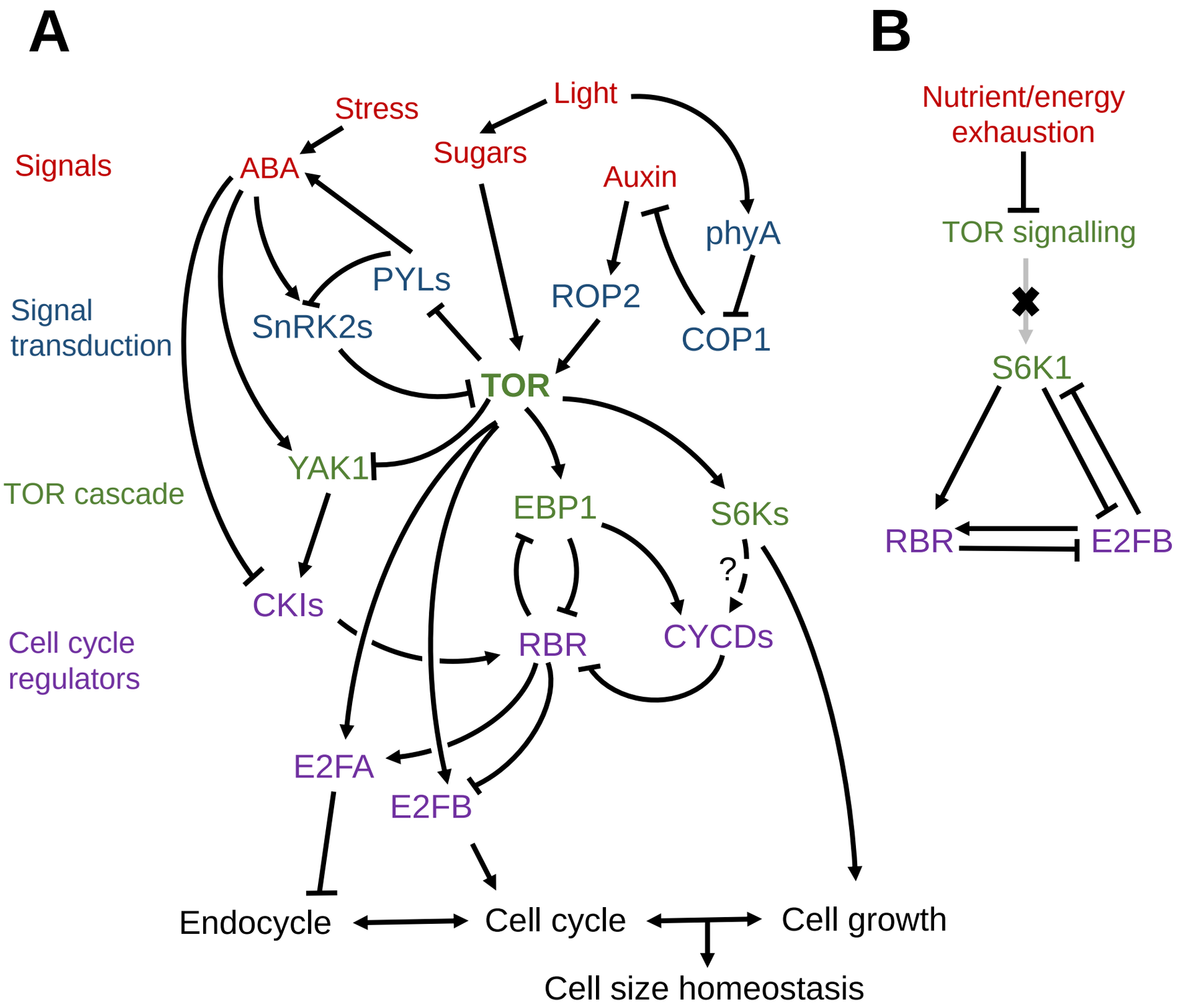
A
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YAK1
EBP1
S6Ks
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RBR
CYCDs
E2FA
E2FB
Endocycle
Cell cycle
Cell growth
Cell size homeostasis
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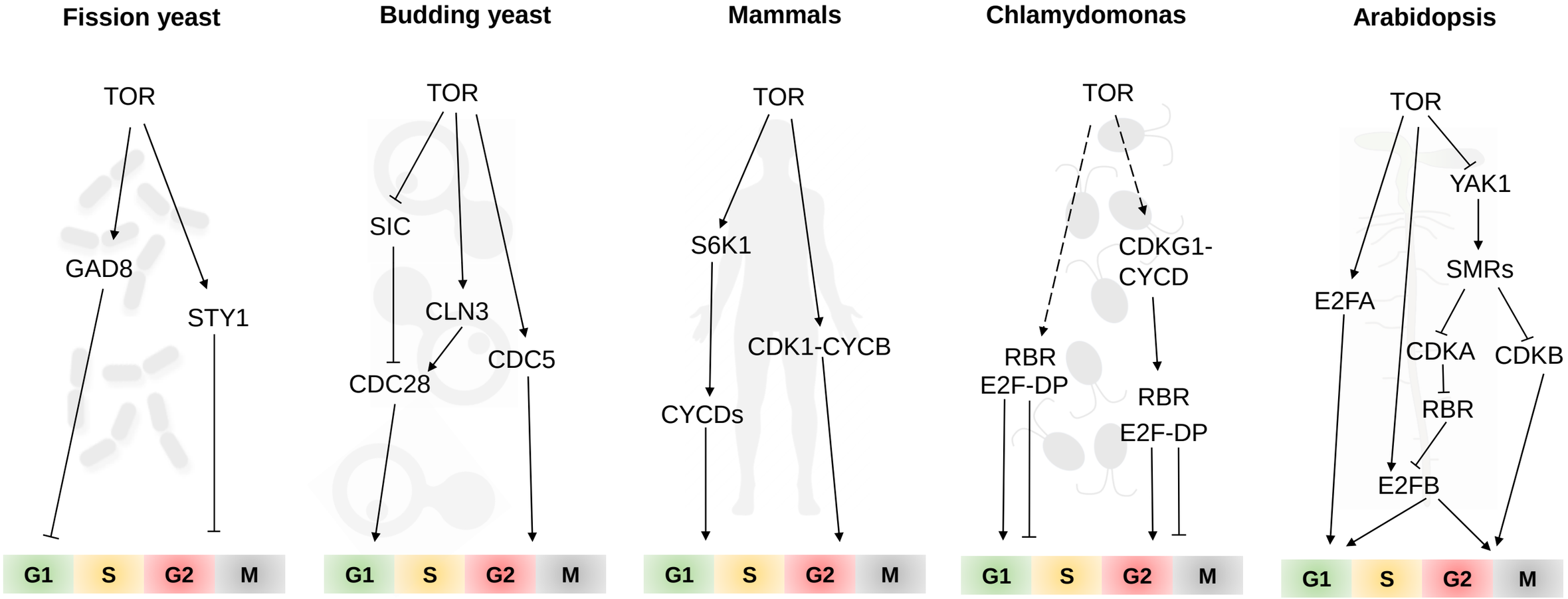
Nutrient/energy exhaustion
TOR signalling
S6K1
RBR
E2FB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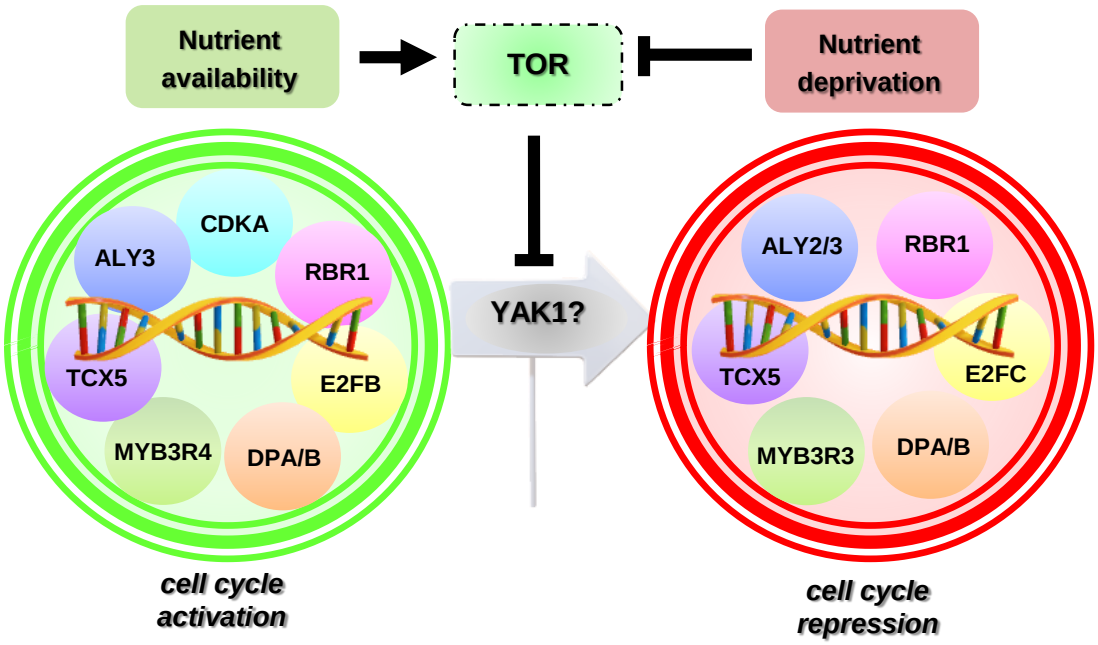
Mammals
TOR
S6K1
CDK1-CYCB
CYCDs
G1
S
G2
M
Chlamydomonas
TOR
CDKG1-
CYCD
RBR
E2F-DP
RBR
E2F-DP
G1
S
G2
M
Arabidopsis
TOR
YAK1
SMRs
E2FA
CDKA
CDKB
RBR
E2FB
G1
S
G2
M


Nutrient availability
TOR
Nutrient deprivation
CDKA
ALY3
RBR1
TCX5
E2FB
MYB3R4
DPA/B
YAK1?
ALY2/3
RBR1
TCX5
E2FC
MYB3R3
DPA/B
cell cycle activation
cell cycle repression