Journal of Experimental Botany, Vol. 65, No. 10, pp. 2691–2701, 2014
doi:10.1093/jxb/eru049 Advance Access publication 24 February, 2014

Journal of Experimental Botany
www.jxb.oxfordjournals.org

REVIEW PAPER

Balancing act: matching growth with environment by the TOR signalling pathway

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Received 28 October 2013; Revised 2 January 2014; Accepted 23 January 2014

Abstract

One of the most fundamental aspects of growth in plants is its plasticity in relation to fluctuating environmental conditions. Growth of meristematic cells relies predominantly on protein synthesis, one of the most energy-consuming activities in cells, and thus is tightly regulated in accordance with the available nutrient and energy supplies. The Target of Rapamycin (TOR) signalling pathway takes a central position in this regulation. The core of the TOR signalling pathway is conserved throughout evolution, and can be traced back to the last eukaryotic common ancestor. In plants, a single complex constitutes the TOR signalling pathway. Manipulating the components of the TOR complex in *Arabidopsis* highlighted its common role as a major regulator of protein synthesis and metabolism, that is also involved in other biological functions such as cell-wall integrity, regulation of cell proliferation, and cell size. TOR, as an integral part of the auxin signalling pathway, connects hormonal and nutrient pathways. Downstream of TOR, S6 kinase and the ribosomal S6 protein have been shown to mediate several of these responses, although there is evidence of other complex non-linear TOR signalling pathway structures.

Key words: Cell proliferation, cell size, growth, nutrient, signalling, TOR.

Introduction

Plant growth occurs through the production of cells at distinct zones and by the elongation of existing cells. Both processes—the continuous addition of new cells and their elongation—also promote the redirection of growth. Therefore, our understanding of plant growth and development relies on several fundamental questions: (1) how cell growth and division are coordinated; (2) how the number of newly produced cells is defined to match the demands set by developmental coordinates, environmental signals, and available nutrients; (3) how the timing of transition from proliferative to elongation growth is set; and (4) how further elongation growth is fuelled and terminated at defined times. In this review, we will discuss recent studies related to basic growth processes, such as protein synthesis and its regulation by signalling pathways that ultimately fine tune cellular growth with available resources. In addition, we will also focus on the coordination of cell growth with cell proliferation in order to maintain cell size homeostasis in meristems and on the importance of breaking this homeostasis during rapid outgrowth of organ primordia.

The principal processes underlying plant growth are well known, conserved during evolution, and covered by recent reviews, such as the mechanism and regulation of protein translation (Bailey-Serres *et al.*, 2009; Roy and von Arnim, 2013), regulation of cell proliferation (De Veylder *et al.*, 2007), and the exit from cell proliferation and onset of cell

Abbreviations: RNAi, RNA interference; uORF, upstream open reading frame.

enlargement (Braidwood *et al.*, 2013). In addition, the basic principles of organ growth in developing leaves have been reviewed extensively by Gonzalez *et al.* (2012). Recent studies have also mapped out the signalling pathways involved in energy sensing and nutrient status that are conserved in plants (Robaglia *et al.*, 2012). There is an emerging importance of global balancing of resources in harmony with anticipated and stressful environmental changes to sustain growth (Smith and Stitt, 2007). In this review, we aim to combine these fields and ideas and focus on the environmental control of growth processes in proliferating cells. For the sake of clarity, we refer to growth as the increase in predominantly cytoplasmic mass, as opposed to turgor-driven cell growth through the uptake of water to vacuoles, which will be referred to as cell elongation. We will focus on growth driving cell proliferation in meristems and young developing organs, especially under the fluctuating environmental conditions that plants continuously experience. This is the case of day/night rhythms, which are less understood and covered, regardless of their importance on C assimilation into biomass (Gonzalez *et al.*, 2012).

Matching growth with environmental conditions through global regulation of translation

Cell growth requires protein synthesis, a process that consumes a major part of the total energy, carbon, and nitrogen pools, and therefore needs to be tightly regulated, so that cellular reserves can match the growth demands (Piques *et al.*, 2009; Sajitz-Hermstein and Nikoloski, 2010). In accordance, the level of protein synthesis closely follows the energy status of the cell, and is responsive to growth-promoting (e.g. light) (Juntawong and Bailey-Serres, 2012; Pal *et al.*, 2013), as well as growth-restricting (e.g. dark, hypoxia, and various stresses) conditions (Branco-Price *et al.*, 2008; Hummel *et al.*, 2010). Protein synthesis is also regulated by the diurnal oscillation of carbon supply, being reduced during the night period (Pal *et al.*, 2013). Similar to protein synthesis, the growth rate of different organs such as leaves (Pantin *et al.*, 2011) and roots (Yazdanbakhsh *et al.*, 2011) also follows a diurnal pattern. Particularly, leaf emergence and early growth rely mostly on cell proliferation and are at peak during the day, in synchrony with the increased protein synthesis rate. In contrast, turgor-driven elongation growth of leaf cells is maximal during the night, a period when water is less limiting (Yazdanbakhsh *et al.*, 2011). Root growth, on the other hand, shows a more complex diurnal change with a sharp peak in the morning after light exposure, followed by a second growth maximum during the night. This might be due to the dual nature of root growth, as it relies on: (1) cell proliferation in the meristem; and (2) cell elongation as cells exit this zone. Importantly, the induction of starvation by extending the night period affects both processes and results in a sudden halt of root growth (Yazdanbakhsh *et al.*, 2011).

Protein translation is initiated with the recruitment of ribosomes to the mRNA to form polysomes (Bailey-Serres *et al.*, 2009; Roy and von Arnim, 2013). In animals, these initial steps are regulated by the evolutionary conserved nutrient-sensing Target of Rapamycin (TOR) signalling pathway, which regulates translation initiation due to its ability to modulate the activity of cap-binding complexes (see below; Serfontein *et al.*, 2010). In plants, however, this is less well understood and appears to occur through different translational regulatory targets (Robaglia *et al.*, 2012).

The Snf1-related AMP-activated kinase (AMPK/SnRK1) signalling pathway regulates cellular energy homeostasis in response to carbon/glucose limitation and stress (Hardie *et al.*, 2012). In animal cells, AMPK represses the nutrient-sensing TOR pathway at multiple points to shut down translation (Hardie *et al.*, 2012). Although this is yet to be demonstrated in plants, the AMPK/SnRK1 phosphorylation site responsible for TOR complex 1 repression (TORC1) is conserved on the plant RAPTOR proteins (Robaglia *et al.*, 2012). Recently, it was shown that the main inputs into the SnRK1 pathway, the glucose level and light, reprogramme cellular metabolism through the TOR pathway (Xiong *et al.*, 2013). The evolutionarily conserved GCN2 protein kinase provides another major route for stress-responsive repression of protein translation (Lageix *et al.*, 2008).

Interestingly, besides its negative effect on protein translation, extension of the dark period is also associated with transcriptional reprogramming, with the induction of a cohort of starvation genes and repression of genes involved in protein translation (Lopez-Juez *et al.*, 2008; Usadel *et al.*, 2008). This suggests that transcriptional and translational responses are co-regulated, possibly by connected signalling pathways. In agreement, it was shown that TOR greatly contributes to switching on of a wide set of glucose-induced genes in *Arabidopsis* (Xiong *et al.*, 2013).

In preparation for protein synthesis, the assembly of ribosomes also poses a large demand on the energy, transcriptional, and translational capacity of cells. Therefore, the number of ribosomes must be finely tuned to match cellular growth with extracellular signals, and nutrient and cellular energy status (Piques *et al.*, 2009; Sajitz-Hermstein and Nikoloski, 2010). Many of the genes coding for ribosomal proteins and other components of the translational machinery are tightly co-regulated, constituting the ribi regulon in yeast (Jorgensen *et al.*, 2004; Loewith and Hall, 2011). Transcripts of ribosomal proteins also co-ordinately increase in growth-promoting conditions in plants, such as in light and at high sugar content (Blasing *et al.*, 2005; Lopez-Juez *et al.*, 2008).

The retinoblastoma protein (Rb) is a central transcriptional repressor that regulates genes required not only for cell proliferation but also for protein translation. In animal cells, it was shown that Rb regulates Pol I and Pol III-dependent transcription (White, 2004), among others, by competing with the activator of the UBF transcription factor on Pol I promoters and affecting rRNA synthesis (Pelletier *et al.*, 2000) while repressing Pol III transcription through TFIIIB and thus tRNA synthesis (White, 2011). UBF is also regulated by phosphorylation at the C-terminal activation domain by S6 kinase 1 (S6K1) in a mammalian (m)TOR-dependent manner in animal cells (Hannan *et al.*, 2003). Interestingly,

AtTOR was also shown to regulate the synthesis of 45S rRNA in *Arabidopsis* (Ren *et al.*, 2011). Besides the involvement of Rb in repressing Pol III-dependent transcription, the MAF1 protein also blocks TFIIIB recruitment to promoters (Cieśla and Boguta, 2008). Interestingly, MAF1 is an ancient, highly conserved protein present in yeast, animals, and plants. Deregulation of protein translation eventually leads to tumour formation and cancer, and this occurs not only in animals but also in plants, where tumour-causing pathogens can boost protein translation by the inactivation of MAF1 (Soprano *et al.*, 2013). These findings further strengthen the notion that uncontrolled protein synthesis can ultimately lead to tumours.

The ErbB3-binding protein-1 (EBP1) belongs to the highly conserved proliferation-associated 2G4 proteins (PA2G4) that bind structured RNAs, possibly linking ribosome biosynthesis and cell proliferation (Squatrito *et al.*, 2004; Kowalinski *et al.*, 2007; Monie *et al.*, 2007). HsEBP1 in humans is both nucleolar and cytoplasmic but has distinct functions in the different cellular compartments (Squatrito *et al.*, 2004, 2006). In the nucleolus, HsEBP1 is part of ribonucleoprotein complexes and associates with different rRNA species. As it is a component of the pre-ribosome subunit, it could act as a cofactor of ribosome biogenesis by regulating pre-rRNA processing (Squatrito *et al.*, 2004). In the cytoplasm, HsEBP1 associates with 40S and 60S ribosomal subunits and is able to inhibit the phosphorylation of the eukaryotic Initiation Factor 2α (eIF2α) by the PKR protein kinase. Thus, HsEBP1 controls protein translation by acting as a cellular inhibitor of eIF2α phosphorylation and promotes the maintenance of protein translation (Squatrito *et al.*, 2006). Although the molecular function for EBP1 is unknown in plants, it was shown to be a dose-dependent growth regulator that might operate through the regulation of protein translation (Horvath *et al.*, 2006).

In summary, these findings strongly suggest that growth is tightly linked with the biosynthesis of macromolecules, mainly proteins. To maintain cellular homeostasis, global regulation of translational capacity and translation initiation are important in order to match the demand for protein synthesis with the nutrient and energy status of cells.

Regulation of growth by selective translation

Regulation of growth can also occur through selective translation of specific mRNAs coding for regulators involved in plant growth and development. This occurs through *cis*-acting elements in mRNAs that regulate translation via upstream open reading frames (uORFs) in the 5′-untranslated region (Bailey-Serres *et al.*, 2009; Roy and von Arnim, 2013). uORFs are particularly prevalent in mRNAs coding for transcription factors involved in growth regulation, signalling molecules, and cell-cycle regulators (Kim *et al.*, 2007). The selective translation of uORF-containing mRNAs coding for basic zipper transcription factors involved in sugar signalling, as well as auxin response factors (ARFs) was shown to rely on the eIF3 non-core subunit h (eIF3h) and the 60S protein, RPL24 (Roy *et al.*, 2010; Schepetilnikov *et al.*, 2013). Auxin activates TOR and consecutively S6 kinase that will phosphorylate eIF3h in order to maintain translation through uORFs in polysomes (Schepetilnikov *et al.*, 2013). In addition, the auxin response-related phenotypes of the mutants, *eif3h*, *rpl24*, *TOR*-RNAi, and the TOR inhibitor-treated plants confirm the functional importance of translation reinitiation in the auxin pathway (Schepetilnikov *et al.*, 2013). In this way, TOR constitutes a convergence point for nutrient, energy, and auxin pathways to regulate growth. However, the exact mechanism and the upstream auxin signalling components that regulate TOR activity are yet to be defined (Bogre *et al.*, 2013).

Effect of ribosome heterogeneity and composition on plant growth and development

The overall organization of the cytosolic ribosome is essentially the same in every eukaryotic organism. In yeast and mammalian cells, each ribosomal protein (r-protein) is typically encoded by a single transcribed gene, although duplications often occur. By contrast, in plants, the number of expressed ribosomal genes varies on a large scale, ranging from two to seven paralogues per family; thus, with 251 r-protein genes in *Arabidopsis*, this gives rise to a staggering number (10^{34}) of different ribosomes (Carroll, 2013). Post-translational modification adds a further level of complexity to the regulation of ribosome function (Carroll, 2013). In fact, proteomics data have confirmed the presence of different r-protein paralogues in distinct ribosomes (Giavalisco *et al.*, 2005; Hummel *et al.*, 2012). Moreover, the variations of the relative abundance and modification states of different r-protein paralogues can impact on physiological, developmental, and growth properties of plants (Carroll, 2013).

There is a growing list of ribosomal gene-related mutants with specific growth and developmental patterning effects, mostly in the shoot and leaf and to a lesser extent in the root (Tsukaya *et al.*, 2013). These defects occur at the level of polarity establishment, in the apical–basal patterning and shape determination of the leaf, and are accompanied by the loss of apical dominance, and disturbed phyllotaxis and cotyledon number. Besides these phenotypic changes, there are mutants with abnormal trichome branching, an indication of changes in the ploidy level, as well as altered sensitivity to auxin, abscisic acid, temperature, and DNA damage agents (Carroll, 2013). The severity of a specific phenotypic change, e.g. abaxialization, appears to be dependent on the identity of the disrupted ribosomal gene, suggesting that different r-proteins have a distinct impact on development. Moreover, these developmental abnormalities depend not only on the qualitative functional difference among paralogues but on their quantity as well. The models describing these phenotypic abnormalities are based on: (1) ribosome insufficiency; (2) ribosome heterogeneity in translational specialization and control; and (3) aberrancy (Carroll, 2013). Plants, therefore, have developed an enormous heterogeneity in ribosomal

composition that will ultimately regulate growth responses and developmental processes.

The evolutionarily conserved TOR complex is required for cell growth, maintenance of meristems, and adjustment of metabolism and carbon balance with plant growth

TOR, a highly conserved serine/threonine kinase of the phosphatidylinositol-3-kinase related family (PIKK), is a master regulator of growth in eukaryotes (Laplante and Sabatini, 2012; Robaglia *et al.*, 2012; Jewell *et al.*, 2013).

TOR signalling has been studied intensively in yeast (Loewith and Hall, 2011) and animal cells (Laplante and Sabatini, 2012; Fig. 1). Here, TOR functions in two distinct complexes, TORC1 and TORC2. TORC1 is rapamycin sensitive and includes TOR, Lethal with Sec Thirteen 8 (LST8), and Regulatory-Associated Protein of TOR (RAPTOR/KOG1), while TORC2 includes TOR, LST8, Raptor Independent Companion of TOR (RICTOR), and Stress-activated MAP Kinase Interacting Protein 1 (SIN1) (Serfontein *et al.*, 2010; van Dam *et al.*, 2011; Beauchamp and Platanias, 2013). Both the upstream regulation and the downstream targets of these two TOR complexes are distinct. TORC1 is part of nutrient-sensing pathways with a major role in the regulation of protein synthesis and cell growth, while TORC2 is activated by growth factors and regulates the cytoskeleton rearrangement, metabolism, and cell-cycle progression (van Dam *et al.*, 2011; Beauchamp and Platanias, 2013).

Although TORC1 and TORC2 complexes are present in a diverse range of organisms, plants only possess components of the TORC1 complex, whereas in some ciliates only TORC2 is present (van Dam *et al.*, 2011; Robaglia *et al.*, 2012).

Evolutionary studies have shown that the central components of the TOR signalling pathway are conserved among divergent species and are suggested to have evolved from an evolutionary core, present in a primitive pathway of the last eukaryotic common ancestor (Beauchamp and Platanias, 2013; Serfontein *et al.*, 2010; Takahara and Maeda, 2013; van Dam *et al.*, 2011). This evolutionary core is comprised of the upstream regulator AMPK (AMP-regulated kinase), TOR, Raptor, and the downstream target S6K (Serfontein *et al.*, 2010). During evolution, new regulatory steps, such as TOR activation by the insulin growth factor pathway in animal cells (Laplante and Sabatini, 2012), or auxin regulation in plant cells (Bogre *et al.*, 2013; Schepetilnikov *et al.*, 2013), were added to provide growth regulation in a multicellular

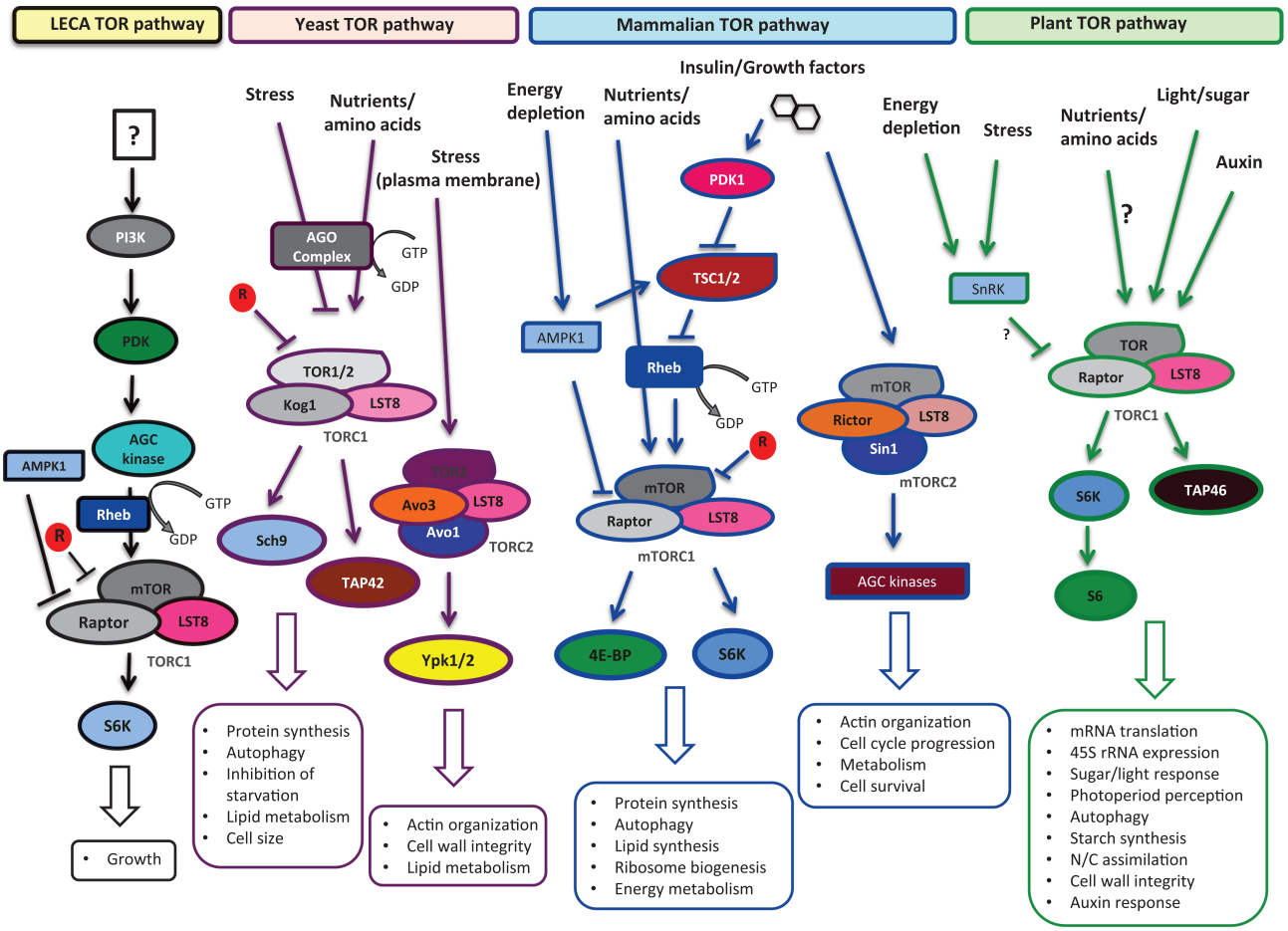

Fig. 1. Evolution of the TOR signalling pathway. The TOR pathway evolved from a core present in the last eukaryotic common ancestor (LECA). The inputs, main components, and outputs of the TOR pathway are shown in different outline colours in yeast (purple), animals (blue), and plants (green). Rapamycin inhibition is depicted by an 'R' in a red circle. See text for more details. (This figure is available in colour at *JXB* online.)

environment. The outputs of the TOR signalling module also diverged through additions to the core, such as the translation control through 4E-BP that exists in animals but not in plants (Robaglia *et al.*, 2012). Other layers of regulation were obtained with duplication events both upstream and downstream of TOR, especially for genes encoding AGC kinases, such as PKB and S6K (Bögre *et al.*, 2003; Pearce *et al.*, 2010).

The *Arabidopsis* genome has a single *TOR* gene, while Raptor and LST8 are encoded by two independent loci (Robaglia *et al.*, 2012). The major known downstream target of AtTOR is S6K, encoded by two genes in linked loci (Mahfouz *et al.*, 2006). The TOR inhibitor rapamycin has aided most functional studies of TOR signalling in yeast and animals. However, plant TOR is largely rapamycin insensitive due to mutations in the FKBP12 protein that prevent the assembly of the inhibitory complex TOR–rapamycin–FKBP12 (Ren *et al.*, 2011, 2012). This rapamycin insensitivity could have been acquired relatively recently in the plant lineage, as *Chlamydomonas reinhardtii* still maintains rapamycin sensitivity at a concentration range similar to that observed in yeast. In agreement with this, the *Chlamydomonas* FKBP12 protein is closer in its sequence to the human and yeast orthologues than to the *Arabidopsis* one (Dobrenel *et al.*, 2011).

The pervasiveness of lethality, combined with AtTOR insensitivity to rapamycin, called for alternative strategies in plants. These strategies comprised: (1) depletion of TOR transcripts by RNA interference (RNAi) or artificial miRNA (Deprost *et al.*, 2007; Caldana *et al.*, 2013); (2) isolation of knockdown mutants affecting different regions of the TOR protein (Ren *et al.*, 2011); (3) inhibition of TOR activity by expression of yeast FKBP12 protein to confer AtTOR sensitivity to rapamycin treatments (Sormani *et al.*, 2007; Leiber *et al.*, 2010; Ren *et al.*, 2012); (4) overexpression of TOR (Deprost *et al.*, 2007); and (5) treatment of *Arabidopsis* plants with high concentrations of rapamycin (Xiong and Sheen, 2012; Xiong *et al.*, 2013) or (6) with novel TOR-specific ATP-competitive inhibitors (Montané and Menand, 2013) (Table 1).

Arabidopsis tor mutant embryos develop, in size and shape, to a stage reminiscent of the dermatogenic stage. However, these *tor* embryos possess more than the normal eight cells, an indication that cell proliferation has continued without cell growth until the developmental arrest, although the reason for this arrest is still unclear (Menand *et al.*, 2002; Robaglia *et al.*, 2012). On the one hand, cells could reach a critical small size due to continued cell proliferation without growth, similar to the described mitotic catastrophe in yeast (Navarro *et al.*, 2012). On the other hand, proliferation without the required enlargement might restrict development by a mechanical signal that halts cell proliferation, characteristic for plant meristems (Hamant *et al.*, 2008) and animal organs (Lecuit and Le Goff, 2007).

Depletion of TOR kinase has resulted in overall smaller plants with a significant reduction in biomass (Deprost *et al.*, 2007; Ren *et al.*, 2011; Ren *et al.*, 2012; Caldana *et al.*, 2013; Montané and Menand, 2013). The mutants developed shorter roots due to reduction of the meristem (Ren *et al.*, 2012) and smaller leaves with smaller epidermis, spongy, and palisade mesophyll cells (Ren *et al.*, 2012; Caldana *et al.*, 2013). Interestingly, the number of palisade cells slightly increased with TOR silencing (Caldana *et al.*, 2013), in agreement with yeast data on TOR's role in inhibiting mitosis to maintain cell size homeostasis (Navarro *et al.*, 2012). Treatments with specific inhibitors also resulted in a shorter root meristem and an elongation zone containing shorter epidermal cells (Montané and Menand, 2013). The reduction in meristem size is the consequence of an early exit from proliferation and entry into the differentiation programme. Although TOR is predominantly expressed in the root and shoot meristems (Menand *et al.*, 2002), compromised TOR function led to shorter hypocotyls in the dark as well as to shorter root hairs. This could indicate that either a low level of TOR is present in these cells during normal development and thus is required for elongation, or that TOR has a non-cell autonomous function, possibly by generating a diffusible signal required for cell elongation. In fact, TOR has been proposed to promote root growth by sensing a glucose signal generated in the shoot, especially at the transition from heterotrophic to photoautotrophic growth (Dobrenel *et al.*, 2011; Xiong *et al.*, 2013). Inhibition of TOR signalling also prevented nutrient-depleted plants from adjusting their metabolism and growth when nutrient levels were restored. These plants were also unable to respond to high light intensity, an indication that TOR is required for adequate perception of nutrient availability and energy levels, which will then result in an adjusted metabolism (Ren *et al.*, 2012).

There is a striking overlap between the phenotype of TOR, RAPTOR, and LST8 mutants, which suggests their function in a common complex. For instance, *Chlamydomonas* LST8 was shown to co-purify with the TOR kinase domain and associate with high-molecular-weight complexes located at the endomembrane system. Similarly, *Arabidopsis* LST8-1 interacts with TOR FKB and localizes to endosomes (Moreau *et al.*, 2012). However, unlike *tor* and *raptor1B*, *lst8-1.1* mutants are viable. Nevertheless, they show reduced growth habit, loss of apical dominance, and an overall bushy appearance due to activation of axillary meristems. Flowering is delayed and developmentally compromised, leading to reduced fertility. These developmental phenotypes become more accentuated in long days, suggesting that lack of LST8-1 affects photoperiod perception. Metabolic profiling of *lst8-1.1* mutants after the transition from short to long days showed accumulation of: (1) nitrate; (2) starch; (3) soluble sugars; and (4) amino acids, with a reduction in raffinose content, suggesting the inability of *lst8-1.1* mutants to mobilize the necessary reserves in order to cope with changes in day length. Interestingly, this metabolic reprogramming highly resembles that of *tor*-depleted plants (Moreau *et al.*, 2012; Ren *et al.*, 2012; Caldana *et al.*, 2013). Transcriptional profiling further confirmed the day-length-dependent phenotype of *lst8-1.1* mutants. Late flowering observed under long days correlated with a reduction in the expression of *Flowering Locus T* (*FT*). The phenotypic alterations due to *LST8-1* depletion strongly suggest that the TOR pathway regulates several transcriptional and metabolic changes required to adjust to longer days.

Table 1. *Summary of experimental approaches designed to modulate the levels of TOR signalling components and the corresponding phenotypes*

Inhibition of TOR pathway	Phenotypes	References
T-DNA insertion, *tor-1*, *tor-2*	Arrested embryos after dermatogen stage; expressed in meristems	Menand *et al.* (2002)
T-DNA insertion, *tor-3* to *tor-14*	Growth defects; embryo lethality; requirement of kinase motifs to rescue embryo lethality	Ren *et al.* (2011)
TOR RNA interference (inducible or constitutive)	Inhibition of growth and mRNA translation with reduction in polysome's accumulation; decreased expression of growth regulator EBP1; regulation of abscisic acid signalling	Deprost *et al.* (2007)
TOR Artificial microRNA	Growth arrest and changes in primary metabolism particularly carbon partitioning and nitrogen metabolism; accumulation of TCA intermediates, starch and triacylglycerides; reprogramming of secondary metabolism	Caldana *et al.* (2013)
Chemical inhibitors	TOR acting as a sensor of glucose levels in shoot to control root meristem size; putative targeting of E2FA to regulate cell division and S-phase gene expression	Xiong and Sheen (2012); Xiong *et al.*, (2012)
ATP-competitive inhibitors	Inhibition of root growth, and smaller cells in meristematic zone that entered early in differentiation without changing their fate; fewer and smaller root hairs	Montané and Menand (2013)
Overexpression of FKBP-12 (rapamycin-sensitive TOR)	Inhibition of root and shoot growth, and decreased mRNA and protein synthesis; reprograming of primary and secondary metabolism; accumulation of starch, triacylglycerides and TCA intermediates; regulation of C partitioning; modification of cell-wall structure with altered expression of expansins and extensins; prolonged life span	Ren *et al.* (2012); Sormani *et al.* (2007); Leiber *et al.* (2010)
Overexpression of TOR motifs (full-length and kinase motif)	Defects in lateral flowering meristem and changes in leaf patterning; kinase domain promotes activation of 45S rRNA; HEAT domain repeats bind to the 45S rRNA promoter	Ren *et al.* (2011)
TOR overexpression due to T-DNA insertion	Promotion of growth in shoot and root; attenuation of osmotic stress	Deprost *et al.* (2007)
T-DNA insertion, *lst8-1.1* and *lst8-1.2*	Reduced growth, loss of apical dominance, bushy appearance and abnormal flower development; similar changes in metabolism to *tor* mutations: (1) higher starch content, (2) decrease in raffinose content, and (3) higher levels of amino acids; reprogramming of metabolome and transcriptome under long days	Moreau *et al.* (2012)
T-DNA insertion, *raptor1(1B)/raptor2(1A)*	Double homozygous are mostly lethal, but a few survivors show a compromised shoot apical meristem and are unable to grow besides the first two leaves; loss of *Raptor 1/1B* was first annotated as embryo lethal although other reports describe *raptor 1/1B* plants as small with root growth defects, bushy appearance and late flowering	Deprost *et al.* (2005); Anderson *et al.* (2005)
T-DNA insertion, *s6a/s6b*	Double homozygous is lethal; double hemizygous plants show growth delay in roots and shoots, consequence of haploinsufficiency; the root growth defect is associated with reduced meristem activity; single mutations lead to: (1) late flowering, (2) an increase in life span, (3) accumulation of the 40S subunit of the ribosome, (4) altered stoichiometry of the 60S/80S ratio, and (5) a decrease in protein synthesis	Creff *et al.* (2010); Ren *et al.* (2012)
T-DNA insertion, *s6k1s6k2* *S6K1*-RNAi	Double homozygous is lethal; double hemizygous plants show: (1) variation in size, (2) overbranched trichomes, (3) larger flowers with a high percentage of unviable pollen, (4) genome instability, and (5) defects in chromosome segregation especially in the generation of male gametes; S6K also involved in repression of cell proliferation, possibly due to interaction with the E2FB–RBR complex and inhibition of E2FB activity	Henriques *et al.* (2010)

RAPTOR associates with TOR and facilitates its binding to and phosphorylation of their substrates (Anderson *et al.*, 2005; Mahfouz *et al.*, 2006). The *Arabidopsis* genome possesses two genes, *RAPTOR1B*, which is highly expressed, and *RAPTOR1A*, whose expression is almost undetectable. In accordance, *raptor1A* mutants develop normally, while *raptor1B* mutants have been characterized either as embryo lethal (Deprost *et al.*, 2005) or viable with compromised

meristem activity, a slower rate of leaf initiation, and loss of apical dominance (Anderson *et al.*, 2005). Double *raptor1B*/*raptor1A* mutants are mostly non-viable and arrested during early embryogenesis (Deprost *et al.*, 2005), although some survivors can be maintained to the stage of seedling development with emergence of the first two leaves (Anderson *et al.*, 2005).

Besides its role in translation and ribosome biogenesis, TOR signalling has a broad range of functions that in yeast and plants include the regulation of cell-wall composition and structure, as well as sensing cell-wall integrity (Leiber *et al.*, 2010). Suppressor screens for the root hair defective mutant *LRR-extensin 1* (*lrx1*) have identified *Repressor of LRX1* (*ROL5*), a gene similar to yeast *Needs Cla4 to survive 6* (*Ncs6p*). Molecularly, ROL5, as its yeast orthologue, encodes a mitochondrial protein required for tRNA modification. Functionally, these findings suggest that a mitochondrial TOR target might participate in the responses to reactive oxygen species and in the modulation of cell walls (Leiber *et al.*, 2010). In agreement, disruption of TOR signalling, in *tor*- and *lst8*-depleted lines, represses the expression of genes encoding regulators of cell-wall expansion such as expansins and extensins (Moreau *et al.*, 2012; Ren *et al.*, 2012). Therefore, TOR acts as a sensor of nutrient and energy levels to promote growth by favouring the increase in cytoplasmic volume and modulating cell-wall structure accordingly.

Downstream of TOR: S6K and S6

TOR, through the adaptor protein RAPTOR, binds to and activates S6K by phosphorylation. The TOR–RAPTOR–S6K association was shown in plants (Mahfouz *et al.*, 2006), and it is also known that S6K becomes phosphorylated, in a TOR-dependent manner, on a conserved site as detected by a phospho-S6K specific antibody (Xiong and Sheen, 2012; Schepetilnikov *et al.*, 2011, 2013). Because S6K is an integral part of an ancient TOR signalling pathway, it is assumed that many of the TOR functions are performed through S6K. The *Arabidopsis S6K* is encoded by two loci, *S6K1* and *S6K2*, located in close proximity in a tandem array. Single mutants are largely normal, while, in contrast to the overlapping phenotypes of *tor*, *raptor1B*/*raptor1A* and *lst8-1.1*, plants with compromised S6K function have distinct phenotypic alterations, indicating a more complex relationship between S6K and TOR than a linear pathway. The double *s6k1s6k2* mutant is gametophytic lethal. Hemizygous *s6k1s6k2*$^{+/+}$ and silenced *S6K1*-RNAi plants do not show any growth retardation. In contrast, they have enlarged flower and trichome branches, infertility, variable growth habit, and developmental abnormalities in leaf shapes, displaying either extremely narrow leaves or large broadened leaves (Henriques *et al.*, 2010). Most of these abnormalities are due to aneuploidy or higher ploidy (Henriques *et al.*, 2010). It is known that inhibition of *RBR* expression can also lead to polyploidization (Johnston *et al.*, 2010). In fact, S6K1, in complex with RBR, potentiates its nuclear localization and the repression of E2FB (Henriques *et al.*, 2010), a transcription factor that positively regulates both the G1/S and the G2/M transitions (Magyar *et al.*, 2005, 2012).

S6K1 and E2FB mutually inhibit their protein accumulation (Henriques *et al.*, 2013), and it is probable that this double inhibition wiring could reinforce the role of S6K as a switch to promote cell growth while simultaneously repressing cell proliferation (Fig. 2). TOR and S6K are also known to inhibit mitosis and thereby regulate cell-size homeostasis in accordance with nutrient levels in both yeast and animal systems (Fingar *et al.*, 2002; Petersen and Nurse, 2007). In response to starvation, AMPK1/SnRK1 is activated, and it also regulates mitosis directly or through the inhibition of TOR in animal cells (Banko *et al.*, 2011).

TOR was also shown to directly phosphorylate E2FA (Xiong *et al.*, 2013), an E2F transcription factor with a dual role: it promotes cell proliferation and meristem maintenance when in complex with RBR but independently of CYCD3;1, but it can also trigger cell growth and endocycle outside the meristem in a RBR-free form (Magyar *et al.*, 2012). Whether and how TOR phosphorylation of E2FA modulates these two functions remains to be shown.

Auxin is a positive regulator of cell division that not only induces *S6K* expression and activity but also activates and promotes TOR's loading into the polysomes. Active TOR phosphorylates S6K1, initiating a signalling cascade that facilitates the translation reinitiation of mRNA templates with uORFs such as *ARF3* (Schepetilnikov *et al.*, 2013). As mentioned above, these findings indicate that regulation of the TOR pathway and auxin signalling are interconnected.

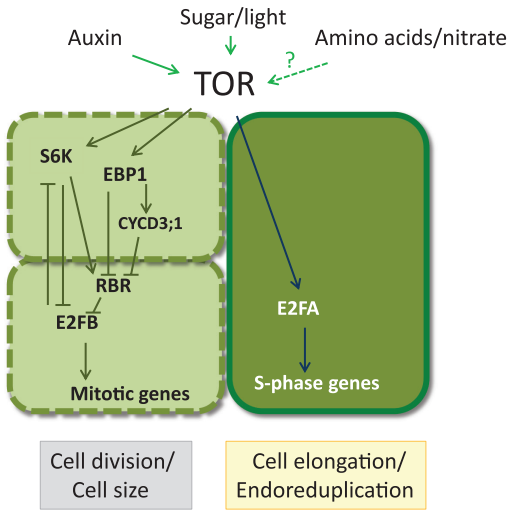

Fig. 2. TOR regulates cell proliferation, cell size, and cell elongation. TOR through S6K potentiates RBR to repress E2FB and thereby cell division (Henriques *et al.*, 2010). As in yeast and animal cells, this might be important to determine cell size (Henriques *et al.*, 2013). TOR was placed upstream of EBP1 (Deprost *et al.*, 2007). EBP1 positively regulates CYCD3;1 and represses RBR (Horvath *et al.*, 2006), and thereby connects cell growth and cell proliferation. TOR can directly phosphorylate E2FA and specifically promote S-phase gene expression (Xiong *et al.*, 2013). The function for this regulation is not clear, but it might uncouple E2FA roles to maintain meristem functions as part of an RBR repressor complex or promote S-phase gene expression and endoreduplication (Magyar *et al.*, 2012). (This figure is available in colour at *JXB* online.)

The ribosomal protein S6 (RPS6) is one of the best-characterized targets of S6K. It is widely accepted that S6 phosphorylation regulates the selective translation of ribosomal proteins leading to the increase in translational capacity. Mutating *rps6* in *Drosophila* inhibits growth in some organs while it promotes cell proliferation in others (Marygold *et al.*, 2007). Yeast *rps6a* or *rps6b* mutants have reduced cell size and growth inhibition (Meyuhas, 2008). Surprisingly, site-directed mutation of the S6K phosphorylation sites on S6 does not compromise protein translation but regulates cell size (Meyuhas, 2008). 4EBPs were also regarded as global translation initiation regulators downstream of TOR, but recent reports have shown that TOR regulates the selective translation of cell proliferation genes through 4EBPs for cell-size control in animal cells (Dowling *et al.*, 2010).

In *Arabidopsis*, *RPS6* is encoded by two genes, *RPS6A* and *RPS6B*, mostly expressed in proliferating cells. The double *rps6a*/*rps6b* mutant is lethal, whereas single *rps6a* or *rps6b* or double hemizygous *rps6a*/*rps6b*$^{+/+}$ plants show reduced root growth and compromised shoots with small pointed leaves (Creff *et al.*, 2010). Genetic interaction studies have shown that inhibition of growth in the TOR-depleted lines requires functional RPS6, indicating that RPS6 would act downstream of TOR (Creff *et al.*, 2010). In addition, downregulation of *RPS6A* or *-B* in TOR-overexpressing plants attenuates the shortened life span and early flowering phenotype typical of these lines, suggesting that RPS6A or -B also participates in the regulation of these processes (Ren *et al.*, 2012). In summary, similar to yeast and animal cells, TOR and S6K signalling has an important role in connecting cell growth to cell proliferation.

Cell-size control

Cell growth and cell division are intimately connected in unicellular organisms in order to attain cell-size homeostasis. In multicellular organisms, however, the connection between cell growth and cell proliferation is more complex and integrated with development. It is debated whether: (1) growth is the major driver being followed by cell proliferation; (2) the two processes are regulated in parallel; or (3) growth is limited by the number of proliferating cells. Interestingly, different sets of data obtained in plants support each of these scenarios. For instance, irradiated wheat seeds where cell division is permanently inactivated are still able to germinate, and seedlings emerge with surprisingly normal-looking leaves and roots, suggesting that cell proliferation is not essential for organ growth (Haber and Foard, 1961; Marshall *et al.*, 2012). In fact, mutations that affect cell proliferation often compensate organ growth by increasing cell size (Horiguchi and Tsukaya, 2011). Similarly, treating meristems with microtubule depolymerization drugs blocked mitosis but not DNA synthesis, hardly affecting meristem growth and differentiation (Grandjean *et al.*, 2004). Surprisingly, blocking DNA synthesis in meristems did inhibit/stop growth, suggesting that cells have to complete S phase and enter into G2 phase in order to grow (Grandjean *et al.*, 2004).

However, to sustain plant development, a continuous supply of proliferating cells is critical, and the number of cells produced during the proliferative stage of organ growth is a pivotal determinant for the final organ size (Gonzalez *et al.*, 2012). Cell production relies on the maintenance of cell proliferation competence in plant meristems. Tracking cells in meristems and in developing organs showed that changes in cell growth behaviour are intimately linked with developmental programmes, especially during organ formation. In the dome of the floral meristem, where cells are maintained in a pluripotent state, growth is slow and isotropic, and cells maintain a constant small size. In contrast to this, in the flanks of the meristem where organ primordium initiates, cell behaviour is different, allowing faster proliferation and increased volume with the consequent cell-size heterogeneity (Reddy and Meyerowitz, 2005; Schiessl *et al.*, 2012).

Although the regulation of cell-size homeostasis is critical for organ growth, the regulatory mechanisms are poorly understood. Lessons from fission yeast (*Schizosaccharomyces pombe*), which mostly grow during the G2 phase of the cell cycle, suggest that the distance from each end of the cells to the nucleus is measured by gradients of mitosis-entry regulating molecules. Budding yeast (*Saccharomyces cerevisiae*) grows predominantly in the G1 phase and relies on a different mechanism, where cytoplasmic volume is measured against a constant entity, the genomic DNA (Jorgensen *et al.*, 2004; Wang *et al.*, 2009). In floral meristems, S-phase labelling and cell-size measurements showed that cells passing the S phase had predominantly larger volumes, suggesting that the cell-size control operates in G1, at which point only sufficiently large cells enter the S phase (Schiessl *et al.*, 2012). This mechanism closely resembles cell-size checkpoints in budding yeast and in animal cells (Jorgensen *et al.*, 2004). In fact, the molecular mechanism underlying G1 cell-size control in budding yeast 'measures' the absolute amount of CLN3, a highly unstable G1 cyclin (proxy for total cytoplasm) against a constant DNA amount (due to the fixed number of CLN3–CDK–SBF-binding sites on genomic DNA). As the CLN3 amount is proportional to cell size, as cells grow it will eventually out-titrate the limiting amount of available SBF-binding sites, at which point cell-cycle entry occurs (Wang *et al.*, 2009). D-type cyclins are the animal and plant orthologues of CLN3, and overexpression of *Arabidopsis CYCD3;1* strongly reduces cell size (Dewitte *et al.*, 2003). On the other hand, E2F transcription factors are analogous to SBF, and indeed both *Arabidopsis* E2FB (Magyar *et al.*, 2005) and *Drosophila* E2F1 (Neufeld *et al.*, 1998) are critical regulators of cell size. In budding yeast, CLN3 acts through the transcriptional repressor Whi5 and the histone deacetylase Rpd3 to regulate the activity of the SBF transcription complex (Wang *et al.*, 2009). Although there are no sequence similarities to the yeast components, the Rb pathway regulation of E2Fs provides an analogous system in animal and plant cells (De Veylder *et al.*, 2007). The unicellular alga *Chlamydomonas* grows up to 30-fold during G1, and this is followed by a rapid proliferation phase of multiple rounds of DNA synthesis and cell divisions, which results in uniformly sized daughter cells (Umen, 2005). Mutating the *Chlamydomonas* orthologue of

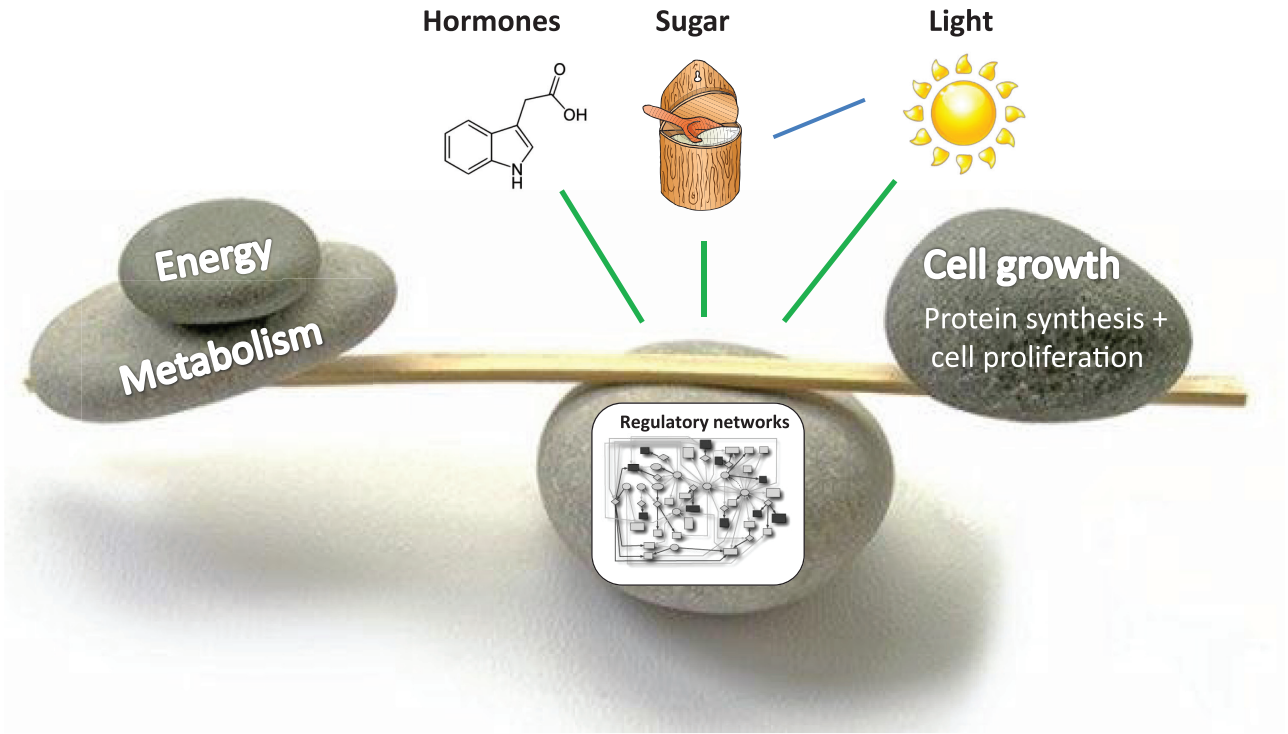

Fig. 3. Balancing regulatory networks for cell growth and metabolism. (This figure is available in colour at *JXB* online.)

RB leads to small-sized cells (Umen and Goodenough, 2001), while mutants in E2F or DP (E2F dimerization partner) are large, clearly demonstrating the role of the RB pathway in cell-size homeostasis (Fang *et al.*, 2006; Fang and Umen, 2008; Olson *et al.*, 2010).

Conclusions

It is of central interest how nutrients, carbon, and energy are converted to plant growth and biomass. Nutrient- and energy-sensing signalling pathways are intermingled with hormonal regulation and cell proliferation control pathways to balance resources with growth, proliferation, and cell elongation (Fig. 3). Past and recent research have made tremendous progress on the understanding of how these different pathways are regulated, by focusing mostly on the model weed *Arabidopsis*. However, now it is time to start testing some of these emerging ideas on crop plants in order to optimize yield. Detailed molecular and phenotypic analysis of maize leaf growth already provides a proof of concept for how leaf growth is underpinned by the hormonal balance to determine cell proliferation and cell elongation transition zones to attain greater biomass and yield (Nelissen *et al.*, 2012).

Acknowledgements

The authors are grateful to the Hungarian Academy of Sciences for inviting and funding the sabbatical stay for LB in the Biological Research Centre, Szeged, in the laboratories of ZM and Ferenc Nagy. The work related to this review is funded by the Ramón y Cajal fellowship from the Spanish Ministerio de Economía y Competitividad (RYC-2011–09220) and by EU Marie Curie Career Integration Grant (PCIG2012-GA-2012–334052) to RH, by the EU Marie Curie Career Development fellowship (FP7-2012 MC-IEF-330789) to BH and LB and by the OTKA 107838 for ZM.

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