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# Glucose–TOR signalling reprograms the transcriptome and activates meristems

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**Meristems encompass stem/progenitor cells that sustain postembryonic growth of all plant organs. How meristems are activated and sustained by nutrient signalling remains enigmatic in photosynthetic plants. Combining chemical manipulations and chemical genetics at the photoautotrophic transition checkpoint, we reveal that shoot photosynthesis-derived glucose drives target-of-rapamycin (TOR) signalling relays through glycolysis and mitochondrial bioenergetics to control root meristem activation, which is decoupled from direct glucose sensing, growth-hormone signalling and stem-cell maintenance. Surprisingly, glucose–TOR signalling dictates transcriptional reprogramming of remarkable gene sets involved in central and secondary metabolism, cell cycle, transcription, signalling, transport and protein folding. Systems, cellular and genetic analyses uncover TOR phosphorylation of E2Fa transcription factor for an unconventional activation of S-phase genes, and glucose-signalling defects in *e2fa* root meristems. Our findings establish pivotal roles of glucose–TOR signalling in unprecedented transcriptional networks wiring central metabolism and biosynthesis for energy and biomass production, and integrating localized stem/progenitor-cell proliferation through inter-organ nutrient coordination to control developmental transition and growth.**

Plant photosynthesis, fuelled by solar energy, $CO_2$ and water to generate renewable organic carbon and oxygen, has a central role in sustaining human life and the ecosystems on Earth. Despite its vital importance, the molecular mechanisms by which photosynthetic products are sensed locally and systematically to activate the metabolic and growth programs in the meristems remain poorly understood[1,2]. TOR kinase is a master regulator evolutionarily conserved from yeasts to plants and human, that integrates nutrient and energy signalling to promote cell proliferation and growth[3–5]. Recent research emphasizes the roles of mammalian TOR kinase in translational controls of cell proliferation[6], insulin signalling[7,8] and cancer initiation and metastasis[9]. In photosynthetic plants, the molecular functions and the dynamic regulatory mechanisms of TOR kinase remain largely unclear, as the embryo lethality of null *Arabidopsis tor* mutants, partial deficiency of inducible *tor* mutants, and the prevailingly perceived rapamycin resistance have hampered genetic and chemical elucidations[3,4,10].

At the onset of plant life, the integrated metabolic and developmental programs switch from heterotrophic utilization of maternal seed reserves to photosynthesis-driven metabolic reprogramming and signalling. This switch allows support of potentially infinite plant growth with renewable carbon and energy production in response to $CO_2$ and sunlight[11–13]. How plant photosynthetic source and sink organs are coordinated to convey nutrient status, what is the nature of nutrient signals, and how meristems are activated and sustained to continuously supply new cells for growth by photosynthesis are all unknown. To begin to address these fundamental questions, we established a simple and sensitive plant system at the transition checkpoint of heterotrophic to photoautotrophic conversion in *Arabidopsis* seedlings[13,14]. We applied a combination of chemical, genetics, genomics, bio-computational and cell-based analyses to dissect the TOR signalling networks in meristem activation and plant growth. We discovered that photosynthesis controlled TOR signalling, which was predominantly stimulated by glucose through glycolysis and mitochondrial bioenergetics relays, to rapidly control metabolic transcription networks and activate the cell cycle in root meristems. Surprisingly, TOR signalling was decoupled from direct glucose sensing via the hexokinase 1 (HXK1) glucose sensor[11], growth-hormone signalling[15] and stem-cell maintenance[2]. Our findings establish an unprecedented molecular framework delineating previously unexpected transcriptional regulation of central and secondary metabolic pathways, biogenesis and key regulators of stem and progenitor cell proliferation by TOR kinase. This TOR-regulated molecular framework provides energy, metabolites, biomass, cell cycle machineries and peptide and redox regulators that concertedly drive stem/progenitor-cell proliferation and plant growth through inter-organ nutrient coordination (Supplementary Fig. 1). Integrative systems, cellular and genetic analyses identified E2Fa transcription factor as a novel TOR kinase substrate for an unconventional activation of S-phase genes in cell cycle entry and a determinant of glucose sensitivity in the root meristem. Plant TOR kinase acts as a gatekeeper gauging and linking the photosynthesis-driven glucose nutrient status to comprehensive growth programs through metabolically-regulated signal transduction and transcriptional networks.

## Photosynthesis controls root meristems

Although photosynthesis and sugars have a decisive role in root meristem activity[13,14], little is known about sugar signalling mechanisms in roots. *Arabidopsis* seedlings germinated in photosynthesis-constrained and sugar-free liquid medium initiated photomorphogenesis, but entered a mitotic quiescent state with arrested root meristem and growth after the depletion of endogenous glucose at 3 days after germination (DAG)[13,14,16] (Fig. 1a–c). Photosynthesis propelled by higher light and ambient CO2 was sufficient to promote a rapid and predominantly root growth (Fig. 1a–d). Using the thymidine analogue 5-ethynyl-2′-deoxyuridine (EdU) for *in situ* detection of cell-cycle S-phase entry[17], we showed that the primary root meristem entered mitotic quiescence after the depletion of maternal nutrient supplies, but could be reactivated by photosynthesis (Fig. 1e). Exogenous glucose (15 mM) taken up by root glucose

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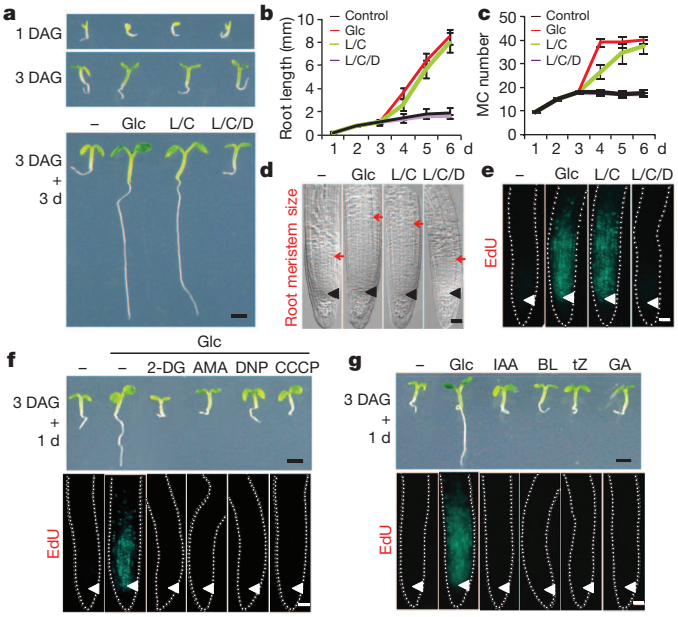


**Figure 1 | Photosynthesis controls the metabolic activation of root meristems.** **a**, Photosynthesis promotes root growth. DAG, day-after-germination, Glc, glucose; L, light; C, $CO_2$; D, DCMU. **b**–**e**, Photosynthesis drives root meristem activation. Results of primary root length, meristem cell (MC) number, meristem size and S-phase entry. Means ± s.d. ($n \geq 25$). **f**, Glycolysis and mitochondrial bioenergetics stimulate root growth and meristem proliferation. 2-DG, 2-deoxyglucose; AMA, antimycin A; DNP, 2,4-dinitrophenol; CCCP, carbonylcyanide-m-chlorophenylhydrazone. **g**, Growth-hormone treatments. IAA, indole-3-acetic acid; BL, brassinosteroid; tZ, trans-zeatin; GA, gibberellins. Scale bar, 1 mm (**a** and **f**, **g**, upper panels) or 25 µm (**d**, **e** and **f**, **g**, bottom panels). Arrowheads, quiescent centre; red arrow, transition zone.

transporters[18] was sufficient to fully substitute for photosynthetic support of root meristem activation and the acceleration of root growth (Fig. 1a–e). The quiescent meristem reactivation occurred within 2 h of glucose treatment (Supplementary Fig. 2). Root growth was quadrupled while the root meristem cell number and size doubled in 24 h (Fig. 1b–g). Consistent with long-distance sucrose and glucose transport through the phloem from shoots to roots[1,19], this inter-organ glucose signalling and growth coordination was completely blocked by the herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) inhibiting photosynthesis (Fig. 1a–e). Only physiological levels of glucose and sucrose, but not other sugars (fructose, xylose and galactose), strongly supported root growth and meristem activation (Supplementary Fig. 3).

Root meristem activation and growth required specific glucose metabolism via glycolysis and mitochondrial electron-transport-chain/oxidative-phosphorylation. The glycolysis blocker 2-deoxyglucose (2-DG), the mitochondrial electron transport inhibitor antimycin A (AMA), and the mitochondria uncouplers, 2,4-dinitrophenol (DNP) and carbonylcyanide m-chlorophenylhydrazone (CCCP)[5], completely prevented both root growth and root meristem reactivation (Fig. 1f). Reactivation of the quiescent root meristem by light and $CO_2$ through photosynthesis also relied on the same glycolysis–mitochondria relays (Supplementary Fig. 4), reinforcing the intimate link between photosynthesis and glucose metabolism in supporting meristem activation and sink organ growth.

Plant growth hormones, including auxin, cytokinins, gibberellins and brassinosteroids, have been implicated in controlling the balance between cell division and differentiation, which determines the root meristem size in the presence of sugars[20,21]. Surprisingly, none of these growth hormones could promote root growth or reactivate the quiescent root meristem at the heterotrophic-to-photoautotrophic transition checkpoint without photosynthesis or exogenous sugars (Fig. 1g). The addition of a physiological mix of amino acids or glutamine also failed to activate the quiescent root meristem (Supplementary Fig. 5). Our results indicate that glucose acts as the pivotal nutrient signal coordinating leaf photosynthesis and root meristems, and provides a fundamental and evolutionarily conserved metabolic platform through glycolysis–mitochondrial energy relays to supply cellular energetic and signalling requirements for root meristem activation and maintenance.

## Glucose–TOR signalling in root meristems

We applied specific chemical inhibitors and chemical genetics to examine the involvement of TOR kinase in root growth and meristem regulation by glucose and photosynthesis[13]. The inducible *tor* mutant had no detectable TOR protein but displayed normal growth during 3 DAG relying on seed nutrients without photosynthesis or exogenous glucose (Supplementary Fig. 6)[13]. However, rapamycin and oestradiol-inducible *tor* mutants blocked the rapid reactivation of the quiescent root meristem in 2 h and the promotion of root growth by glucose at the photoautotrophic transition checkpoint at 3 DAG (Fig. 2a and Supplementary Fig. 2). Based on rapamycin-sensitive phosphorylation of T449 in S6K1 as a conserved indicator of endogenous TOR kinase activity[13], we revealed that glucose activation of TOR kinase also depended on glycolysis-mitochondria-mediated energy and metabolic relays (Fig. 2b). Significantly, 2-DG, AMA, rapamycin and the *tor* mutant similarly inhibited glucose or light/$CO_2$ promotion of the doubling of root meristem length and cell number, and *de novo* DNA synthesis visualized by EdU *in situ* staining in 24 h (Fig. 2c, d and Supplementary Figs 3, 4, 7a and 8). Importantly, the *Arabidopsis* glucose sensor HXK1 mutant *gin2* did not prevent the glucose-dependent increase of the meristem cell numbers and *de novo* DNA synthesis (Fig. 2a and Supplementary Figs 7b and 9), which is consistent with the uncoupled signalling and catalytic functions of HXK1[11].

## Specificity of glucose–TOR signalling

To evaluate the physiological and signalling impact of blocking the mitochondrial electron-transport-chain and TOR kinase by AMA and rapamycin, respectively, in the root meristem, we examined growth-hormone signalling and stem-cell maintenance using well-established marker genes and reporters[2,15]. Surprisingly, mitochondrial energy relays and TOR kinase activity were decoupled from growth-hormone signalling triggered by auxin and cytokinin (Fig. 3a–d and Supplementary Fig. 10). Quantitative real-time reverse transcriptase PCR (qRT–PCR) analysis revealed that different functional classes of

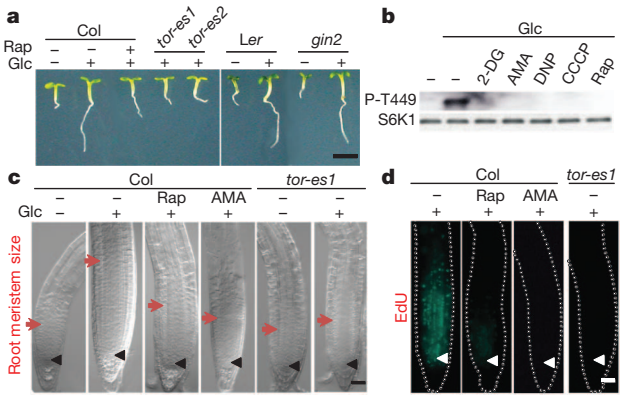


**Figure 2 | Glucose–TOR signalling in root meristems.** **a**, Root growth activation by glucose is TOR dependent. WT (Col or L*er*), oestradiol-inducible *tor* mutants (*tor-es1*, *tor-es2)* and *gin2* seedlings at 3 DAG were incubated without or with glucose (Glc) or rapamycin (Rap) for 24 h. Scale bar, 2 mm. **b**, Glucose activates endogenous TOR. TOR activation is detected using an anti-phospho-T449 antibody for S6K1 after 1 h glucose treatment without or with glycolysis inhibitor (2-DG), mitochondrial blockers (AMA, DNP, CCCP) or rapamycin (Rap). **c**, **d**, TOR controls root meristem activation and S-phase entry. Scale bar, 25 µm.

primary auxin and cytokinin marker genes were all actively induced by hormones in the presence of rapamycin or AMA in wild type, or *tor* seedlings (Fig. 3a, c and Supplementary Fig. 10). Furthermore, rapamycin, AMA or the *tor* mutant did not compromise the activation of auxin and cytokinin signalling green fluorescent protein (GFP) reporters, *DR5::GFP* and *TCS::GFP*, in root meristems (Fig. 3b, d), or perturb the maintenance of the stem-cell niche visualized with the stem-cell and quiescent centre markers, *PLT1::GFP* and *WOX5::GFP*, respectively (Fig. 3e, f). These results unexpectedly indicate that glucose–TOR signalling does not indiscriminately affect general signalling, transcription and translation, but specifically targets cell-cycle regulation of the stem/progenitor cells in the root meristem (Supplementary Fig. 11). Thus, the glucose–TOR signalling network in cell cycle control and root meristem activation is strictly dependent on the glycolysis–mitochondrial energy relays, whereas signalling by plant growth hormones and stem-cell niche maintenance seem to rely on segregated cellular energy and metabolism supporting distinctive transcriptional and translational processes.

## Glucose–TOR directs transcriptional networks

To better understand the molecular landscape of the glucose–TOR signalling networks, we performed genome-wide expression profiling to investigate the rapid global transcriptome changes by 2-h glucose (15 mM) treatment in wild-type and *tor* seedlings at the photoautotrophic transition checkpoint. Primary and dynamic glucose response genes were defined by established microarray data analysis algorithms and filtering[22], and validated by qRT–PCR analyses of marker genes (Fig. 4, Supplementary Methods and Supplementary Tables 1–5). Based on relatively stringent statistics and filtering (both RMA and dChip, $P$ value $< 0.01$; signal ratio change $\log_2 \geq 1$, see Supplementary Methods for details), we defined 1,318 up- and 1,050 downregulated *Arabidopsis* genes differentially controlled by a physiological level of glucose (Fig. 4a, c and Supplementary Table 1). The grand scope of reproducible gene expression changes indicated that glucose directs specific and significant transcriptional networks. Strikingly, this swift global transcriptional reprogramming induced by glucose is completely blocked in the inducible *tor* mutant (Fig. 4a, c). It appears crucial to probe the complex and dynamic glucose–TOR-mediated transcriptome in a bioenergetically quiescent checkpoint with minimal background and growth defects before adding specific TOR stimulating signals and inhibitors in multicellular organisms.

Hierarchical clustering analysis of glucose–TOR target genes with ATH1 GeneChip data sets generated by independent research laboratories revealed significant positive correlation of glucose- and sucrose-regulated genes in seedlings[23,24]. Moreover, adult leaf transcriptome analysis at compensation point [$CO_2$] (50 p.p.m.) limiting photosynthesis[25] confirmed a negative correlation (Fig. 4a, c). These findings further demonstrated that glucose signal is the main nutrient mediator derived from source leaf photosynthesis for systematic gene regulation and root growth. The sensitivity of our system facilitated the discovery of previously unknown primary glucose target genes, especially enriched in cell cycle and DNA synthesis, transcription, and RNA synthesis/processing among glucose-activated genes (Fig. 4a, b and Supplementary Table 2), and modulating transcription, protein degradation and signalling among glucose-repressed genes (Fig. 4c, d and Supplementary Table 2).

Remarkably, the primary glucose–TOR target genes stratify into a myriad of regulatory and metabolic functional categories, including genes that specifically promote proliferation of stem/progenitor cells for root meristem activation and growth (Fig. 4b, d and Supplementary Tables 1, 3, 4, 6 and 7). Glucose–TOR signalling activated genes encoding root growth factor (RGF) peptides[26] (Supplementary Fig. 12) and promoting S-assimilation and glutathione synthesis[27] (Supplementary Figs 12 and 14), all essential for cell proliferation in the root meristem. *UPB1* (UPBEAT1) transcription factor, whose overexpression inhibits root meristem expansion through redox control, was repressed[20] (Supplementary Fig. 12). Significantly, genes (105) important for cell cycle and DNA synthesis (Mapman[28]) are highly activated (Fig. 4b and Supplementary Tables 1, 3 and 6).

Over 100 *Arabidopsis* genes encoding ribosomal proteins and protein synthesis machineries were activated by glucose–TOR signalling (Fig. 4b and Supplementary Tables 1 and 3), supporting a universal TOR function in controlling translational processes[4,5,10,29]. Genes encoding the entire *Arabidopsis* glycolysis and the tricarboxylic acid (TCA) cycle, mitochondrial activities and the electron transport chain were activated by glucose–TOR signalling (Fig. 4b and Supplementary Fig. 13), suggesting a positive feedback loop in TOR-mediated transcriptional control of central carbon and energy metabolism, which is partially conserved in plants, yeasts and mammals[5,29–31]. TOR kinase also activated genes involved in other key and evolutionarily conserved anabolic processes, including amino acid, lipid and nucleotide synthesis and the oxidative pentose phosphate pathway, that are essential for rapid growth (Fig. 4b and Supplementary Tables 1 and 3), but repressed genes mediating the degradation of proteins, amino acids, lipids and xenobiotic, and autophagy regulation[32] (Fig. 4d and Supplementary Tables 1 and 4).

Unique to plant glucose–TOR signalling was its pivotal roles in repressing the metabolic genes for enzymes involved in β-oxidation and glyoxylate cycle required in the germination program of *Arabidopsis* seeds[12], and suppressing catabolic programs for plant survival in the prolonged darkness[32] (Fig. 4d and Supplementary Tables 1 and 4). Glucose–TOR signalling also activated broad gene sets coding for the synthesis and modification of plant cell walls, cell-wall proteins (arabinogalactan proteins and expansins), lignin, pectin, secondary metabolites, and a large variety of small molecules[28] (Fig. 4b and Supplementary Tables 1 and 3). Notably, key *MYB28/34* transcription factors for the synthesis of glutathione and the indolic/benzoic and aliphatic glucosinolate synthesis pathways (Supplementary Fig. 14), and the genes for lignin and flavonoid synthesis pathways (Supplementary Fig. 15) were also activated by glucose–TOR signalling. Coupled with the extensive TOR regulation of carbon metabolism and biosynthesis was the TOR activation of a large set of genes for protein folding (heat-shock proteins, chaperones and prefoldins), nutrient/metabolite transporters (nitrate transporter and glucose-6-phosphate translocator), lipid transfer proteins, protein secretion and targeting, and vesicle trafficking, but downregulation of genes for various sugar transporters (STP and SWEET)[1,18] (Supplementary Tables 1, 3 and 4). The plant glucose–TOR signalling networks also integrated a large number of transcription factors, chromatin modulators, signalling regulators and growth- and stress-related proteins that could be unique to plants or conserved in eukaryotes (Fig. 4b, d and Supplementary Table 1). Our findings

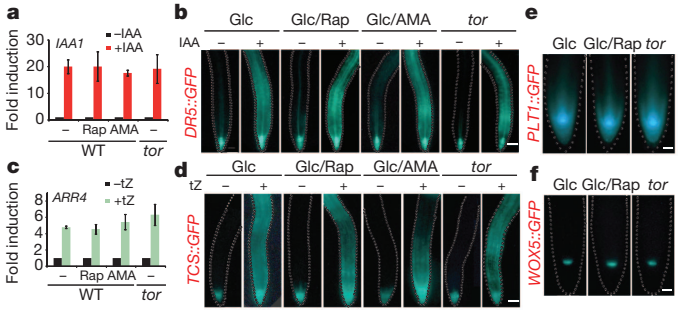


**Figure 3 | Auxin and cytokinin signalling and root stem-cell maintenance are decoupled from TOR activation.** **a**, **b**, Auxin signalling. **c**, **d**, Cytokinin signalling. Rapamycin (Rap), mitochondrial blocker (AMA). Primary auxin and cytokinin marker genes were activated by 1 h of indole-3-acetic acid (IAA) or trans-zeatin (tZ) treatment, and analysed by qRT–PCR. Means ± s.d., $n = 3$. *DR5::GFP* or *TCS::GFP* was activated by 6 h of IAA or tZ treatment. Scale bar, 50 μm. **e**, **f**, Root stem-cell maintenance is TOR independent. *PLT1::GFP*, root stem-cell marker; *WOX5::GFP*: root quiescent centre marker. Scale bar, 20 μm.

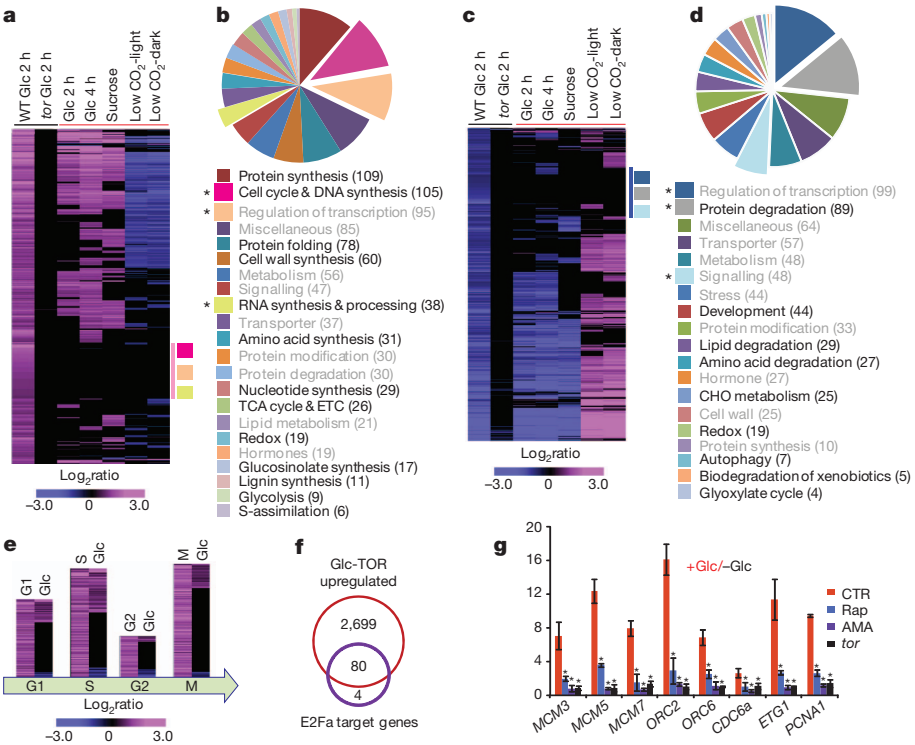


**Figure 4 | Glucose–TOR signalling orchestrates transcriptome reprogramming.** **a**, **b**, Glucose–TOR-activated genes. **c**, **d**, Glucose–TOR-repressed genes. 3 DAG WT or *tor* seedlings were treated without or with glucose (Glc) for 2 h. Hierarchical clustering analysis of glucose–TOR genes and five independent data sets (Glc 2 h, Glc 4 h, sucrose, low $CO_2$-light, low $CO_2$-dark). Deep-pink/blue bar indicates novel glucose–TOR genes. The enriched functional categories are highlighted in bold (Supplementary Tables 1, 3 and 4). ETC, electron transport chain. CHO, carbohydrate. **e**, Hierarchical clustering analysis of glucose–TOR genes (Glc) and cell cycle genes (G1, S, G2, M)[33]. **f**, Glucose–TOR-activated genes overlap with E2Fa target genes. **g**, Glucose–TOR activates S-phase genes. qRT–PCR analyses. Means ± s.d., $n = 3$. $^*P < 0.05$.

uncover a previously unanticipated central role of TOR in glucose and energy signalling through rapid transcriptome reprogramming, which is beyond the conventional emphasis on translational controls for mammalian TOR kinase via 4E-BP and S6K[5,6,9].

## Novel glucose–TOR–E2Fa regulatory relay

To further explore the molecular mechanism by which glucose–TOR signalling controls cell proliferation for meristem activation and root growth, we compared our glucose–TOR target genes with reported cell-cycle oscillation genes[33] using relaxed stringency (both RMA and dChip, $P$ value $< 0.01$) (Supplementary Table 5). Hierarchical clustering analysis revealed that many glucose–TOR-activated genes matched the typical G1- and S-phase genes (Fig. 4e and Supplementary Table 6). As E2F transcription factors are conserved key regulators of S-phase genes governing cell cycle progression and DNA replication in plants and mammals, we performed stringent computational analyses to identify putative *Arabidopsis* E2Fa target genes, which were defined by *E2Fa* co-expression (Genevestigator), activation by E2Fa induction in transgenic plants, and possessing putative E2F-binding sites in promoter regions[34–36] (Supplementary Fig. 16 and Supplementary Table 7). A subset of glucose–TOR-activated genes notably overlapped (95%) with the putative *Arabidopsis* E2Fa target genes (Fig. 4f). Glucose rapidly activated *ORC2/6* (*ORIGIN RECOGNITION COMPLEX*), *MCM3/5/7* (*MINOCHROMOSOME MAINTENANCE*), *CDC6* (*CELL DIVISION CYCLE*), *ETG1* (*E2F TARGET GENE*) and *PCNA1* (*PROLIFERATING CELL NUCLEAR ANTIGEN*), which were significantly diminished in the *tor* mutants or by rapamycin, 2-DG or AMA treatment in wild-type seedlings (Fig. 4 g and Supplementary Fig. 18), but not in the glucose sensor *gin2* mutant (Supplementary Fig. 17). Consistently, glucose or sucrose but not other sugars activated these E2Fa target genes (Supplementary Fig. 18), indicating that the dynamic glucose–TOR signalling may partially execute its cell proliferation regulation through E2Fa transcription factor.

E2Fs are the well-established targets of the universal CYC-CDK-RBR (CYCLIN-CYCLIN-DEPENDENT KINASE-RETINOBASTOMA-RELATED PROTEIN) cascade initiating cell cycle[33–36]. To explore the novel regulatory link between TOR kinase and E2Fa, we developed a sensitive cell-based assay, in which ectopic expression of E2Fa alone was sufficient to activate S-phase-specific marker genes in non-dividing and fully differentiated leaf cells (Fig. 5a). S-phase gene activation by E2Fa and T449 phosphorylation in S6K1 were inhibited by rapamycin, AMA or the *tor* mutant (Fig. 5a). Significantly, immunoprecipitated endogenous TOR kinase from *Arabidopsis* plants directly phosphorylated E2Fa *in vitro* (Fig. 5b), which was completely blocked by a specific ATP-competitive TOR kinase inhibitor, torin1[37] (Fig. 5b). Consistently, torin1 inhibited T449 phosphorylation of S6K1 *in vivo* and S-phase gene activation by E2Fa in non-dividing leaf cells (Supplementary Fig. 19). This plant TOR kinase also phosphorylated the human 4E-BP1 *in vitro* (Fig. 5b), and thus seemed to resemble the rapamycin-sensitive mammalian-TOR-complex1 (mTORC1) but not mTORC2[4,6,10]. Based on the differential specificity of PK inhibitors[7,8,37], we further demonstrated that E2Fa was a direct substrate of TOR kinase but not the TOR-activated S6K1, which could be inhibited by staurosporine but not torin1 (Fig. 5c). As a direct substrate, E2Fa co-immunoprecipitated with TOR in cells (Fig. 5d). These results indicate that direct E2Fa protein phosphorylation by TOR kinase may be a key step for glucose activation of S-phase genes bypassing or acting downstream the conventional CYC-CDK-RBR cascade.

To map the TOR kinase phosphorylation region(s) in E2Fa, various truncated E2Fa proteins were generated, including the amino-terminal putative regulatory, DNA-binding-dimerization, and the carboxy-terminal transcription activation/RBR-interacting domains[33–36] (Fig. 6a). The *in vitro* kinase assay revealed that the TOR kinase phosphorylation site(s) are located in the N-terminal 80-residue regulatory domain (Fig. 6a). Surprisingly, removal of the previously defined C-terminal transcription-activation/RBR-interacting domain did not abolish E2Fa activation of S-phase target genes, whereas deletion of the N-terminal TOR kinase phosphorylation region rendered E2Fa inactive without affecting protein translation/stability (Fig. 6a, b). Interestingly, the DNA binding domain alone without TOR phosphorylation was sufficient as the full-length E2Fa for binding to the predicted E2Fa-binding motifs located in the *MCM5* and *ETG1* promoters based on real-time chromatin-immunoprecipitation-qPCR (ChIP-qPCR) analyses (Fig. 6c). These results indicated a novel mechanism of TOR phosphorylation in regulating the activity of E2Fa in transcriptional activation, probably independent of S6K, RBR or translational control (Figs 5 and 6a–c).

The Pro-rich 80 residues contained 16 Ser/Thr residues that could potentially serve as TOR phosphorylation sites[7,8] (Supplementary Fig. 20). Systematic mutagenesis analyses of the 16 Ser/Thr in eight clusters (Supplementary Fig. 20a) did not reveal dominant TOR kinase phosphorylation sites for the E2Fa activity in target gene activation, suggesting combinatorial or redundant TOR phosphorylation illustrated by mammalian 4E-BP1 and Grb10[5,7,8]. The mutation of all 16 Ser/Thr residues significantly diminished E2Fa activity (Supplementary Fig. 20b).

To substantiate the genetic link and independently evaluate this surprising glucose–TOR-E2Fa signalling cascade in root meristem activation, we screened and isolated a null allele of the *e2fa* mutant (Supplementary Fig. 21). The truncated E2Fa protein failed to activate target genes (Supplementary Fig. 21c). In the absence of glucose, no overt difference was observed between wild type and *e2fa* in root length and meristem cell number. In contrast, glucose-promoted root growth, root meristem expansion and EdU staining were all significantly compromised in *e2fa* (Fig. 6d and Supplementary Fig. 22a, b). However, other related E2Fs might provide partially overlapping functions[34,35] (Supplementary Fig. 22c, d), which will require detailed investigations. Several reported *e2fa* RNA interference and insertion mutants independently confirmed similar root meristem and growth defects[38]. qRT–PCR analysis demonstrated that *e2fa* displayed specifically diminished glucose sensitivity in TOR activation of S-phase genes in the root meristem (Fig. 6e), providing compelling genetic evidence for a key role of E2Fa, together with RGFs and UPB1, in the glucose–TOR transcriptional networks governing root meristem activation (Fig. 6f).

## Discussion

Comprehensive chemical, genetic, genomic and systems analyses in *Arabidopsis* seedlings at the photoautotrophic transition checkpoint with minimal TOR signalling background was crucial to lead to our discovery of previously unexpected glucose–TOR transcriptional networks. These networks dynamically repress the transcription programs associated with seed nutrient metabolism for germination, and simultaneously stimulate and sustain the meristem activity for infinite root growth through photosynthesis-driven glucose–TOR signalling. Although TOR was first discovered in yeast, the atypical fermentation lifestyle evolved opposite transcriptional regulation of glycolytic and TCA-cycle genes by yeast TOR1[30]. Limited evidence supports mammalian TOR signalling in direct transcriptional control[39]. Current studies emphasize translational regulation by mammalian

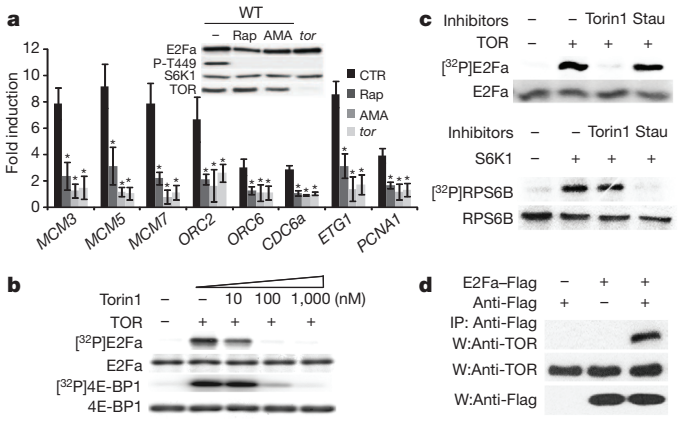


**Figure 5 | TOR kinase phosphorylates and activates E2Fa.** **a**, Ectopic E2Fa activation of S-phase genes requires glucose–TOR signalling in leaf cells. WT or *tor* protoplasts expressing E2Fa–HA or S6K1–Flag were treated without or with rapamycin (Rap) or antimycin A (AMA). qRT–PCR analyses. P-T449 indicates endogenous TOR kinase activity. Protein blot analysis (inset). Means ± s.d., $n = 3$. $^*P < 0.05$. **b**, **c**, TOR kinase directly phosphorylates E2Fa and 4E-BP1. Torin1 specifically inhibits TOR kinase. Staurosporine (Stau) inhibits S6K1 kinase. **d**, TOR directly interacts with E2Fa by immunoprecipitation (IP) and Western (W) blot analysis.

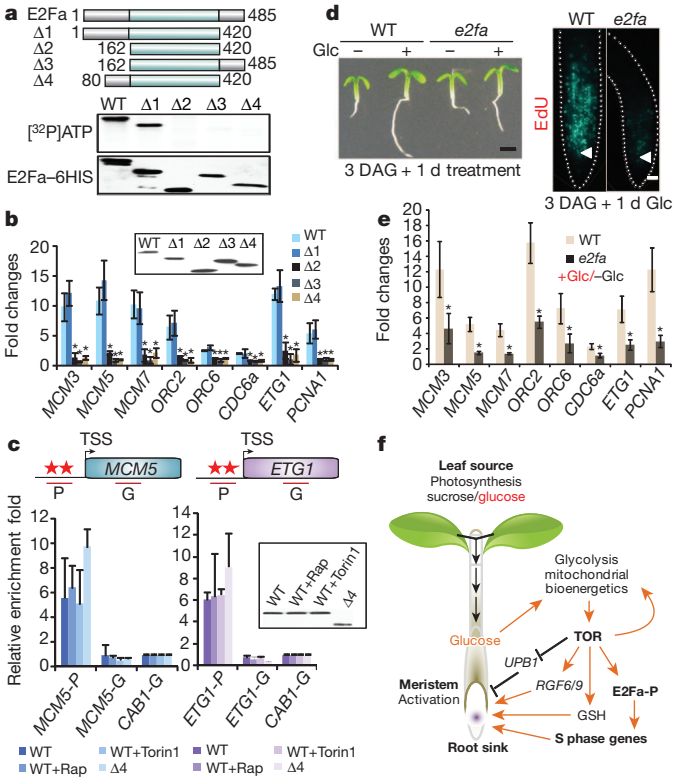


**Figure 6 | TOR kinase controls the activity of E2Fa in transcriptional activation.** **a**, TOR kinase phosphorylates the N-terminal domain of E2Fa. **b**, TOR kinase phosphorylation is critical for E2Fa activation of S-phase genes. **c**, E2Fa-DNA binding is not affected by TOR kinase phosphorylation. ChIP-qPCR analyses with P (promoter) or G (gene body) primers. Stars, putative E2Fa-binding motifs. Error bars ($n = 2$). **d**, **e**, Glucose responses is diminished in *e2fa* root meristems. Scale bar, 1 mm (left) or 20 μm (right). qRT–PCR analyses. **f**, Model of leaf-root coordination in glucose–TOR signalling. GSH, GLUTATHIONE; *RGF*, *ROOT GROWTH FACTOR*; *UPB1*, *UPBEAT1*. Means ± s.d., $n = 3$. $^*P < 0.05$.

TOR via 4E-BP1 and S6K1 phosphorylation, which indirectly modulate limited messenger RNAs and target genes[5,6,9,31]. Unravelling the plant glucose–TOR signalling networks in metabolic and cell-cycle controls may illuminate the unexplored mTORC1 transcription networks for inter-organ nutrient coordination in animals or in human cancers.

TOR signalling has mainly been linked to amino-acid sensing and insulin/growth regulator signalling to modulate translational controls in mammals[5,7–9]. As glucose is a universal fuel and metabolic/biomass precursor for most cells, glucose activation of TOR kinase as a central transcriptional regulator of gene sets involved in glycolysis, TCA cycle, ribosome biogenesis, and the synthesis of proteins, amino acids, lipids and nucleotides are probably conserved in multicellular eukaryotes from plants to humans. Our findings establish a molecular framework for future exploration of transcriptional regulators as new TOR kinase substrates coordinating the genes participating in the most conserved and central metabolic pathways in bioenergetics and biosynthesis fundamental to all multicellular organisms. Glucose–TOR signalling also controls plant-specific genes that are uniquely required for plant growth, defence or communication to promote fitness, adaptation and survival. The molecular wiring of the ancient TOR signalling network controls both conserved and divergent metabolic pathways supporting the diverse lifestyle of different organisms.

We discovered E2Fa as a novel TOR kinase substrate, which transcriptionally activates S-phase genes as primary glucose–TOR target genes. The finding breaks the conventional concept of cell-cycle regulation based on the evolutionarily conserved CYC-CDK-RBR-E2F cascade[34,35]. The direct TOR-E2F link may provide an alternative

entry point of the cell cycle through glucose signalling in the meristem of other plant organs and in other eukaryotes. The successful identification of transcription factors as direct TOR kinase substrates offers an innovative approach for future discovery of unconventional TOR kinase substrates with complex and combinatorial phosphorylation sites. Interestingly, plant growth hormones activate transcription and translation, but are ineffective in mediating cell proliferation in the absence of glucose–TOR signalling (Figs 1g and 3). We propose that glucose–TOR signalling provides essential energy, metabolites, biomass and cell-cycle machineries through concerted transcriptional activation in stem/progenitor cells (Figs 4, 5 and 6). This may explain the prerequisite, fundamentally indispensible and global roles of glucose–TOR signalling in proliferation and growth. Endogenous plant hormones, signalling in specific cells and contexts (Fig. 3)[2,15], may modulate specific cell cycle regulators and bring cell-cycle connections to patterning and developmental programs when nutrients and glucose–TOR signalling are available. The findings on glucose–TOR signalling unravel a missing link in nutrient regulation of meristems in plant growth.

## METHODS SUMMARY

**Quiescent root meristem analyses.** To define the transition checkpoint of heterotrophic to photoautotrophic conversion, root meristem size and cell number were carefully monitored daily under microscope for 6 days (Leica, DM5000).

**Gene expression analysis.** Global gene expression analyses were performed with *Arabidopsis* ATH1 GeneChip arrays (Affymetrix). For details on data processing and analyses, see Supplementary Information.

**Full Methods** and any associated references are available in the online version of the paper.

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**Supplementary Information** is available in the online version of the paper.

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**Author Contributions** Y.X. and J.S. initiated the project and designed the experiments; Y.X. carried out most of the experiments; L.L. and Y.X. conducted quantitative ChIP-PCR analyses; Y.X., M.M. and J.S. analysed the microarray data. C.X. isolated the *e2fa* mutant. Q.H. generated *PLT::GFP* and *WOX5::GFP* transgenic lines. Y.X., M.M. and J.S. wrote the manuscript.

**Author Information** All microarray data are available at the Gene Expression Omnibus under accession number GSE40245. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to Y.X. (xiong@molbio.mgh.harvard.edu) or J.S. (sheen@molbio.mgh.harvard.edu).

## METHODS

**Plant growth conditions.** If not otherwise indicated, all plant materials were grown in a plant growth room with conditions maintained at 23 °C, 65% humidity and 75 μmol $m^{-2}$ s light intensity under 12 h light/12 h dark photoperiod.

**Plant materials.** Col-0 and L*er* were used as wild-type *Arabidopsis* plants. *PLT1::GFP*, *WOX5::GFP* and *gin2* are in L*er* background. All other transgenic plants are in Col-0 background. Oestradiol-inducible *tor* RNAi lines and S6K1-HA overexpression lines were described previously[13]. To generate transgenic *WOX5::GFP* and *PLT1::GFP* lines, the 4.7-kilobase (kb) *WOX5* (*At3g11260*) promoter region and 4.5-kb *PLT* (*At3g20840*) promoter region, respectively, were cloned into an expression vector derived from the pCB302 minibinary vector to drive HXK1–GFP expression. *DR5::GFP*, *TCS::GFP*, *WOX5::GFP* and *PLT1::GFP* lines[15] were crossed with *tor-es1* to generate *DR5::GFP/tor*, *TCS::GFP/tor*, *WOX5::GFP/tor* and *PLT1::GFP/tor* lines. Oestradiol (10 μM) was used to induce TOR depletion, which was confirmed by a specific *Arabidopsis* TOR antibody[13]. The *e2fa* mutant was isolated and confirmed from the *wiscDsLox434F1* line.

**Analyses of root meristem reactivation and root growth.** *Arabidopsis* seeds (6 seeds per well) were germinated in 6-well plates containing 1 ml of glucose-free liquid medium (0.5× MS, pH 5.7 adjusted with KOH) for 3 days to enter the mitotically quiescent state. Quiescent seedlings were treated with glucose (15 mM), plant hormones, amino acid mix (0.1 mM/each) or glutamine (0.1 mM) for the indicated time to reactivate the quiescent root meristem. The concentrations of plant hormones were chosen based on their ability for promoting cell cycle: indole-3-acetic acid (IAA, 0.5 nM)[40], trans-zeatin (tZ, 100 nM)[15,40], gibberellins (GA, 2 μM)[41], and brassinosteroid (BL, 0.01 nM)[42]. Amino acid mix contains 17 amino acids including alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine and cystine. Amino acids failed to activate quiescent root meristem even with high concentration: amino acid mix (1 mM each) or glutamine (0.5 mM and 5 mM, data not shown).

**Chemical inhibitor treatments.** Quiescent seedlings were pretreated with rapamycin (10 μM), AMA (5 μM), 2-DG (15 mM), DNP (50 μM) or CCCP (10 μM) for 1 h before other treatments. The rapamycin effect is facilitated in the liquid medium[13].

**Enhanced photosynthesis assays.** For analysing the effect of photosynthesis on root growth and meristem establishment, quiescent seedlings in glucose-free liquid medium 3 DAG were transferred to glucose-free solid medium (0.5× MS and, pH 5.7, 1% agarose) without/with DCMU (20 μM), and grown vertically at 23 °C under constant light conditions of 300 μmol $m^{-2}$ s light intensity for 3 days. To study the molecular link between photosynthesis and glucose–TOR signalling, the quiescent WT or *tor* seedlings were incubated in 400 μl glucose-free liquid medium (0.5× MS and 6 mM $Na_2CO_3$, pH 5.7) in 6-well plates without/with DCMU (20 μM), 2-DG (15 mM), AMA (5 μM), or rapamycin (10 μM), for 24 h with 200 μmol $m^{-2}$ s light intensity.

**Analyses of auxin and cytokinin signalling.** For activation of auxin and cytokinin primary marker genes, quiescent seedlings were pretreated without/with rapamycin (10 μM) or AMA (5 μM) for 1 h. Glucose (15 mM) was then added for 2 h, followed by IAA (100 nM) or tZ (100 nM) for an additional 1 h. To analyse *DR5::GFP* and *TCS::GFP* reporter lines, quiescent seedlings were pretreated without/with rapamycin (10 μM) or AMA (5 μM) for 1 h. Glucose (15 mM) was then added for 18 h, followed by IAA (100 nM) or tZ (100 nM) for an additional 6 h (for a total of 24 h incubation). For the inducible *tor* mutant, oestradiol (10 μM) was added at the beginning of germination[13].

**Analyses of stem-cell maintenance.** For analysing root stem cells and the quiescent centre, *PLT1::GFP* and *WOX5::GFP* transgenic seedlings were germinated without/with rapamycin for 3 days in 0.5× MS medium with 15 mM glucose.

**Protoplast transient expression assay.** Protoplast transient expression assays were carried out as described[13,15,22,32]. Data were generated from at least three independent experiments with consistent results. Protoplasts ($10^5$) were transfected with 5 μg E2Fa or its truncated variants and incubated for 4 h in 5 ml of mannitol (0.5 M) and KCl (20 mM) buffer (4 mM MES, pH 5.7) in Petri dish (100 mm × 20 mm) for gene activation analysis. For experiments analysing the effect of rapamycin, AMA and torin1 on E2Fa activated S-phase gene expression, protoplasts were pretreated without/with rapamycin (1 μM), AMA (5 μM) or torin1 (100 nM) for 1 h before E2Fa transfection.

**EdU (5-ethynyl-2′-deoxyuridine) staining.** EdU staining was performed as described[17] using EdU detection cocktail (Invitrogen). Briefly, seedlings were treated with 1 μM EdU for 30 min and fixed in 4% (w/v) formaldehyde solution in PBS solution with 0.1% Triton X-100 for 30 min. Fixer was washed with PBS (3 × 10 min) then incubated in EdU detection cocktail for 30 min in the dark, followed by PBS wash (3 × 10 min) before observation by microscope.

**Microscopy and imaging.** All images were recorded with a Leica DFC digital camera mounted to a Leica DM5000 microscope using an FITC (fluorescein isothiocyanate)-specific filter (EdU and GFP) or a DIC (differential interference contrast) filter (transparent roots in 89% lactic acid) except for Supplementary Fig. 9a, in which images were collected with an Olympus FV-1000 confocal microscope with a 488 nm Argon laser (GFP).

**RT–PCR analyses.** Total RNA was isolated from seedlings with TRIzol reagent (Invitrogen) except for the *e2fa* mutant analysis (Fig. 6e), in which total RNA was isolated from the root meristem (0.5 mm root tips). First strand cDNA was synthesized from 1 μg of total RNA with M-MLV (Moloney murine leukemia virus) reverse transcriptase (Promega). All qRT–PCR analyses were performed by CFX96 real time PCR detection system with iQ SYBR green supermix (Bio-Rad). *TUB4 (At5g44340)* and *EIF4a (At3g13920)* were used as control genes.

**Microarray analyses.** Details for microarray data set sources and data analyses are described in Supplementary Methods. Raw CEL files and RMA $\log_2$ signal intensity files are available at Gene Expression Omnibus (GSE40245).


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