



Glucose-TOR signaling regulates PIN2 stability to orchestrate auxin gradient and cell expansion in *Arabidopsis* root

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Edited by Natasha V. Raikhel, Center for Plant Cell Biology, Riverside, CA, and approved October 22, 2020 (received for review July 22, 2020)

The plant growth hormone auxin controls cell identity, cell division, and expansion. In the primary root of *Arabidopsis* there is a robust auxin gradient with a peak concentration at the tip of the meristem and a significant decrease throughout the elongation zone. The molecular mechanisms of how such a steep auxin gradient is established and maintained, and how this auxin gradient within the root dynamically adjusts in response to environmental stimuli are still largely unknown. Here, using a large-scale *Arabidopsis* mutant screening, we described the identification of PIN2 (PIN-FORMED 2), an auxin efflux facilitator, as a key downstream regulator in glucose-TOR (target of rapamycin) energy signaling. We demonstrate that glucose-activated TOR phosphorylates and stabilizes PIN2 and therefore influences the gradient distribution of PIN2 in the *Arabidopsis* primary root. Interestingly, dysregulation of TOR or PIN2 disrupts the glucose-promoted low auxin region located in the elongation zone that is essential for cell elongation. Taken together, our results shed light on how carbon and metabolic status can be tightly integrated with the hormone-driven processes to orchestrate complex plant growth programs.

auxin | glucose | target of rapamycin | root | PIN2

The evolutionarily conserved target of rapamycin (TOR) kinase acts as a master regulator that coordinates cell proliferation and growth by integrating nutrient, energy, hormone, and stress signals in all eukaryotes (1, 2). In plants, TOR senses both the glucose energy signal and the light-auxin hormone signal to promote the rapid plant growth (3–5), although the key downstream effectors are still largely unknown. Our prior studies found that *Arabidopsis* seedlings germinated in photosynthesis-restrained (with limited CO₂) and sugar-free liquid medium entered a mitotic quiescent state with arrested primary root growth due to the depletion of endogenous sugars (4). Exogenously supplied glucose reactivated TOR activity and promoted rapid primary root growth, and this promotion could be abolished by specific TOR inhibitors (e.g., Torin2) (3, 6) (Fig. 1 *A* and *B*). Taking advantage of this quantitatively tractable root growth phenotype, we carried out a large-scale mutant screen using collections of confirmed T-DNA insertion lines from the *Arabidopsis* Biological Resource Center to identify the potential downstream regulators that mediate glucose-TOR promotion of root growth. We isolated one mutant line, SALK_122916c, as it was largely resistant to the inhibition effect of Torin2 on glucose-promoted primary root growth (Fig. 1 *A* and *B*). The T-DNA was inserted in the first exon of *At5g67090*, which was previously designated as *PIN-FORMED 2* (*PIN2*). To exclude possible pleiotropic effects of T-DNA insertion, we examined another well-recognized *PIN2* mutant, *eir1-1* (7), and found that *eir1-1*, as compared to WT seedlings, was also less sensitive to Torin2-inhibited root growth (Fig. 1 *A* and *B*).

We next investigated how *eir1-1* was involved in the glucose-TOR regulation of primary root growth. Previous work has shown

that TOR transduces a glucose energy signal to activate cell proliferation in the root meristem. Unexpectedly, using the thymidine analog 5-ethynyl-29-deoxyuridine (EdU) for in situ detection of cell division activity, we found that TOR inhibition could still significantly abolish the glucose-promoted cell division in *eir1-1*, similar to the response in WT plants (Fig. 1 *C* and *D*).

The *Arabidopsis* primary root can be divided into the meristem, elongation, and differentiation zones. The root length is mainly determined by the cell-proliferation activities within the meristem zone and the final cell length within the differentiation zone. We analyzed the cell length within the differentiation zone in *eir1-1* and WT root, with or without glucose and Torin2 treatments. Compared to WT plants, *eir1-1* is less sensitive to Torin2- or glucose starvation-inhibited cell elongation within the differentiation zone (Fig. 1 *E* and *F*). Together, these data suggested that glucose-TOR signaling controls both cell proliferation and cell elongation in the primary root growth, whereas the resistance to TOR-inhibition phenotype in the *eir1-1* mutant was specifically caused by the cell elongation, but not cell-division activity.

PIN2 functions as an auxin efflux facilitator mediating proximal shootward auxin transport in the *Arabidopsis* root (8). By analyzing the auxin response reporter line *DR5v2::ntdTomato* (9), we found that in the presence of a glucose supply, there was an auxin-response gradient within the primary root (Fig. 2 *A* and *B*). The highest *DR5v2::ntdTomato* signal observed is in the meristem zone (high auxin region, M zone), followed by an abruptly lower signal within the elongation zone and the beginning of the differentiation zone (low auxin region), and then a significantly increased signal in the middle of the differentiation zone (moderate auxin region). Interestingly, glucose depletion or TOR inhibition resulted in only a very narrow low auxin region adjacent to the meristem zone in WT roots, while the remaining elongation zone and neighboring differentiation zone still showed a relatively high auxin signal (Fig. 2 *A* and *B*). In contrast, this narrow auxin-response region under glucose starvation or TOR inhibition was completely blocked in *eir1-1* (Fig. 2 *A* and *B*), indicating an essential role of *PIN2* in this dynamic glucose-TOR regulated auxin gradient establishment.

We then investigated the underlying mechanism by which *PIN2* is involved in glucose-TOR regulation of the auxin gradient

Author contributions: X.Y., P.X., and Y.X. designed research; X.Y., P.X., and Y.Y. performed research; X.Y., P.X., Y.Y., and Y.X. analyzed data; and P.X. and Y.X. wrote the paper.

The authors declare no competing interest.

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First published December 7, 2020.

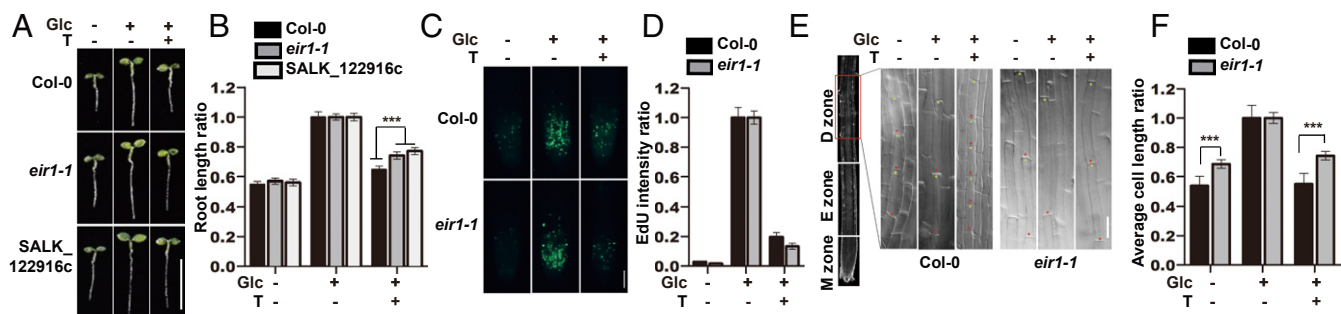


Fig. 1. PIN2 is a key downstream regulator in glucose-TOR signaling. (A) Images of representative seedlings in WT (Col-0), *eir1-1*, and SALK_122916c lines with or without glucose (Glc) and Torin2 (T) treatment. (Scale bar, 5 mm.) (B) Relative ratio of root length of A. Means \pm SE, unpaired two-tailed *t* test, ****P* < 0.001. (C) EdU staining of root meristem in WT (Col-0) and *eir1-1* with or without 2 h glucose (Glc) and Torin2 (T) treatment. (Scale bar, 50 μ m.) (D) Quantification of relative EdU intensity in C. (E) Cell length in differentiation zone of Col-0 and *eir1-1* with or without glucose (Glc) and Torin2 (T) treatment. Images shown are part of the 10th to 16th epidermal cells after onset of cell elongation. (Scale bar, 50 μ m.) D, differentiation; E, elongation; M, meristem. Red and yellow asterisks indicate the bottom and upper boundary of a cell, respectively. (F) Relative ratio of root cell length in E. Means \pm SE, unpaired two-tailed *t* test, ****P* < 0.001.

by analyzing the *pPIN2::PIN2-GFP* transgenic reporter line (10). In the presence of a glucose supply, PIN2-GFP localized apically in the epidermis, with a physical PIN2-GFP gradient distribution

pattern of the highest expression within the root meristem zone followed by modest expression in the elongation zone, and a gradual decrease and disappearance of PIN2-GFP in the

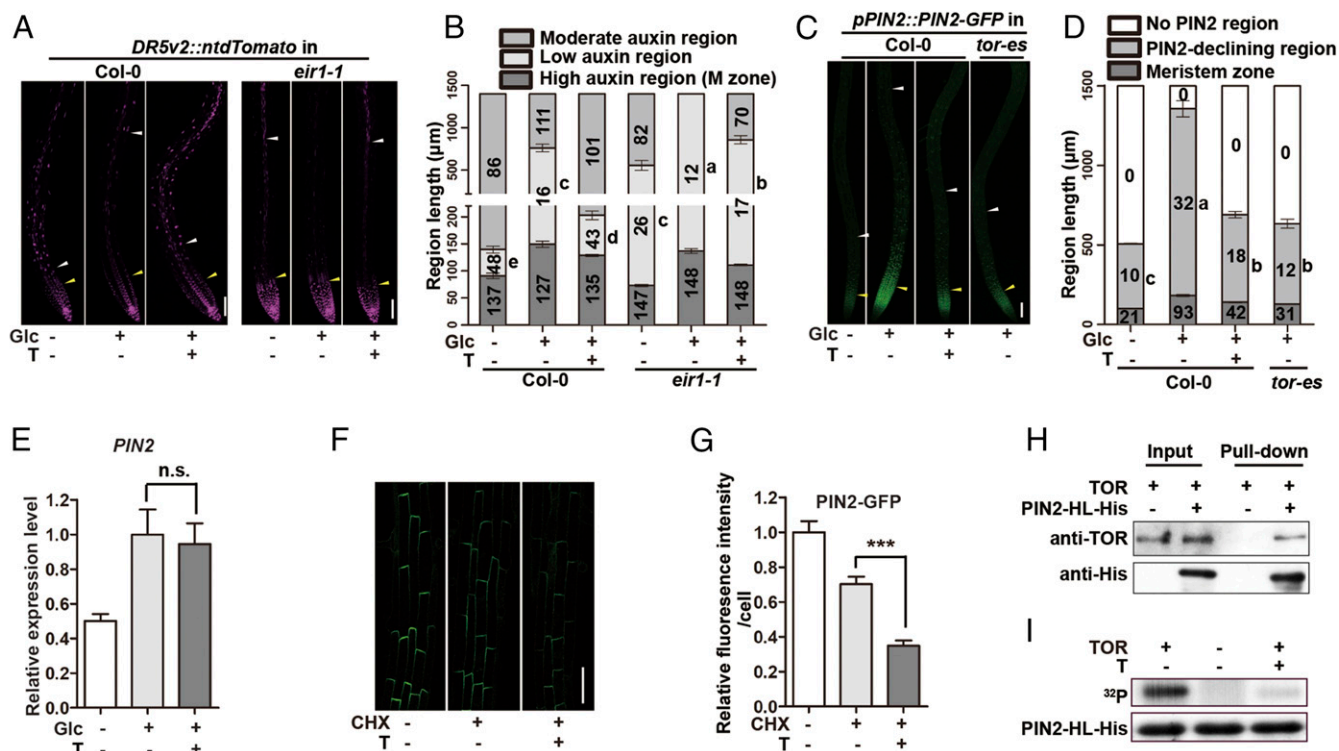


Fig. 2. Glucose-TOR modulates auxin and PIN2 distribution regions in root. (A) Expression patterns of *DR5v2::ntdTomato* in Col-0 and *eir1-1* with or without glucose (Glc) and Torin2 (T) treatment. Yellow arrowheads indicate the upper edge of the meristem; white arrowheads indicate the boundary of low auxin region. Maximal projections of z-stacks are presented. (Scale bars, 50 μ m.) Each whole root image was assembled by two overlapped confocal images. (B) Quantification of auxin-response regions in A. High auxin region (meristem zone, below the yellow arrowheads), low auxin region (between the yellow and white arrowheads), and moderate auxin region (above the white arrowheads). Means \pm SE; *n* \geq 8; the letters beside the bars indicate significant different length of low auxin region under different treatments; unpaired two-tailed *t* test, *P* < 0.05. The numbers inside the bars indicate the fluorescence intensity of each region. (C) Expression patterns of *pPIN2::PIN2-GFP* in Col-0 and *tor-es* with or without glucose (Glc) and Torin2 (T) treatment. The yellow arrowheads indicate the upper edge of the meristem and the white arrowheads indicate the boundary where the expression of PIN2 disappears. Maximal projections of z-stacks are presented. (Scale bar, 50 μ m.) Each whole root image was assembled by three to four overlapped confocal images. (D) Quantification of PIN2 expression regions in C. Meristem zone (below the yellow arrowheads), PIN2-declining region (between the yellow and white arrowheads), and no PIN2 region (above the white arrowheads). Means \pm SE; *n* \geq 8; the letters beside the bars indicate significant different lengths of PIN2-declining region under different treatments, unpaired two-tailed *t* test, *P* < 0.05. The numbers inside the bars indicate the fluorescence intensity of each region. (E) The *PIN2* expression level under glucose starvation or TOR inhibition conditions. Total RNA was isolated from primary root, and analyzed by qRT-PCR. Means \pm SE; *n* = 3; n.s., no significant difference. (F) TOR inhibition triggers a faster PIN2 degradation. The 4-d-old *pPIN2::PIN2-GFP* seedlings grown in the presence of a glucose supply were treated with or without Torin2 (T) and CHX for 3 h. Images shown are cells from the elongation zone. (Scale bar, 50 μ m.) (G) Quantification of relative fluorescence intensity in F. Means \pm SE; *n* \geq 8, unpaired two-tailed *t* test, ****P* < 0.001. (H) TOR interacts with PIN2-HL, revealed by semi-in vitro pull-down assay. HL, hydrophilic loop (amino acids 188 to 477). (I) TOR phosphorylates PIN2-HL, revealed by the in vitro kinase assay.

differentiation zone (Fig. 2 C and D), consistent with prior reports (11). We found that glucose depletion or TOR inhibition (by Torin2 treatment or in estradiol-inducible RNAi *tor* transgenic plants [*tor-es*]) down-regulated the PIN2-GFP level in the elongation and differentiation zones, while the PIN2 polarized location was still maintained (Fig. 2 C and D). Interestingly, Torin2 treatment did not affect the mRNA level of *PIN2* (Fig. 2 E), but triggered a faster PIN2 degradation in *Arabidopsis* primary root in the presence of the protein synthesis inhibitor cycloheximide (CHX) (Fig. 2 F and G), indicating posttranslational regulation of PIN2. Moreover, endogenous TOR kinase immunoprecipitated from *Arabidopsis* seedlings using a TOR-specific antibody directly interacted and phosphorylated the central hydrophilic loop domain of PIN2 (PIN2-HL) (Fig. 2 H and I). Together these results indicate that PIN2 is a substrate of TOR, and that TOR can phosphorylate and stabilize PIN2.

Auxin forms a steep gradient to maintain stem cell identity in the quiescent center, activate cell proliferation in root meristem, and promote cell expansion in the elongation zone, respectively (12). It is well known that very low auxin concentrations stimulate root elongation, while minute increases in auxin levels can quickly inhibit root elongation (13). Our results suggested that glucose-TOR signaling is essential for maintaining such a low auxin response region within the elongation zone in order to promote cell expansion by regulating the expression of the auxin efflux facilitator PIN2. Auxin can be transported from the meristem through the elongation zone to the differentiation zone via PIN2-mediated shootward polar auxin transport to form a high/low-moderate auxin concentration pattern in the primary root. We propose that when glucose-TOR signaling is inhibited, the region with polarized PIN2 in the elongation zone is largely decreased

and, therefore, auxin transport from the elongation zone to the differentiation zone is blocked, leading to a high auxin accumulation in the elongation zone and expansion inhibition in the *Arabidopsis* root.

Taken together, the data in our study provide an example of how dynamic metabolic energy inputs could influence the pre-established hormone signaling to orchestrate complex growth programs in plants. In the future, identification and functional analyses of the TOR phosphorylation sites in PIN2 will help to uncover additional roles of the glucose-TOR-PIN2 axis in plant growth and development.

Methods

Plant Materials and Growth Conditions. All plant materials were grown in a plant growth chamber at 23 °C light/21 °C dark, 65% humidity, and 75 $\mu\text{mol}/\text{m}^2$ s light intensity under 16-h light/8-h dark photoperiod. *Arabidopsis* seeds were germinated in six-well plates containing 1 mL of glucose-free liquid medium (1/2 MS, pH = 5.7) for 4 d to enter the mitotically quiescent state. Quiescent seedlings were treated with 15 mM glucose for 1 d with or without Torin2 (0.25 μM , pretreated for 1 h) or estradiol (1 μM , pretreated for 2 d) to reactivate root growth. For monitoring the PIN2 degradation rate, 4-d-old seedlings grown in the presence of a glucose (15 mM) supply were treated with Torin2 (0.25 μM) and CHX (50 μM) for 3 h.

EdU staining assay, semi-in vitro pull-down assay, and in vitro kinase assay was performed as described previously (4).

Data Availability. All study data are included in the article.

ACKNOWLEDGMENTS. We thank Shingo Nagawa for kindly providing us the *DR5v2::ntdTomato*, *eir1-1*, and *DR5v2::ntdTomato/eir1-1* seeds. The research included in this report is supported by National Natural Science Foundation of China Grant 31870269 (to Y.X.), and the Basic Forestry and Proteomics Research Center, Haixia Institute of Science and Technology, Fujian Agriculture and Forestry University.

1. G. Y. Liu, D. M. Sabatini, mTOR at the nexus of nutrition, growth, ageing and disease. *Nat. Rev. Mol. Cell Biol.* **21**, 183–203 (2020).
2. L. Fu, P. Wang, Y. Xiong, Target of rapamycin signaling in plant stress responses. *Plant Physiol.* **182**, 1613–1623 (2020).
3. X. Li *et al.*, Differential TOR activation and cell proliferation in *Arabidopsis* root and shoot apices. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 2765–2770 (2017).
4. Y. Xiong *et al.*, Glucose-TOR signalling reprograms the transcriptome and activates meristems. *Nature* **496**, 181–186 (2013).
5. L. Bögre, R. Henriques, Z. Magyar, TOR tour to auxin. *EMBO J.* **32**, 1069–1071 (2013).
6. Q. Liu *et al.*, Characterization of Torin2, an ATP-competitive inhibitor of mTOR, ATM, and ATR. *Cancer Res.* **73**, 2574–2586 (2013).
7. C. Luschnig, R. A. Gaxiola, P. Grisafi, G. R. Fink, EIR1, a root-specific protein involved in auxin transport, is required for gravitropism in *Arabidopsis thaliana*. *Genes Dev.* **12**, 2175–2187 (1998).
8. M. Adamowski, J. Friml, PIN-dependent auxin transport: Action, regulation, and evolution. *Plant Cell* **27**, 20–32 (2015).
9. C. Y. Liao *et al.*, Reporters for sensitive and quantitative measurement of auxin response. *Nat. Methods* **12**, 207–210 (2015).
10. J. Xu, B. Scheres, Dissection of *Arabidopsis* ADP-RIBOSYLATION FACTOR 1 function in epidermal cell polarity. *Plant Cell* **17**, 525–536 (2005).
11. L. Abas *et al.*, Intracellular trafficking and proteolysis of the *Arabidopsis* auxin-efflux facilitator PIN2 are involved in root gravitropism. *Nat. Cell Biol.* **8**, 249–256 (2006). Corrected in: *Nat. Cell Biol.* **8**, 424 (2006).
12. R. Di Mambro *et al.*, Auxin minimum triggers the developmental switch from cell division to cell differentiation in the *Arabidopsis* root. *Proc. Natl. Acad. Sci. U.S.A.* **114**, E7641–E7649 (2017).
13. H. Motte, S. Vanneste, T. Beeckman, Molecular and environmental regulation of root development. *Annu. Rev. Plant Biol.* **70**, 465–488 (2019).