Faculty Opinions

ConducTORs of a Signaling Symphony: Metabolic and Hormone Responses Converge on TOR and EIN2 in plants

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EVALUATION OF

Landmark

The TOR-EIN2 axis mediates nuclear signalling to modulate plant growth.

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Development is coordinated by dozens of signals that act in overlapping pathways to orchestrate multicellular growth. Understanding how signaling pathways intersect and diverge at a molecular level is critical to predicting how organisms will react to dynamic environmental conditions. In plants, two antagonistic signaling hubs are strictly required to sense and respond to many nutrients and hormones: TARGET OF RAPAMYCIN (TOR) and ETHYLENE INSENSITIVE 2 (EIN2). In this Landmark report, Fu *et al*. discover that TOR and EIN2 directly interact to choreograph growth and define an unexpected molecular mechanism at the intersection of hormonal and metabolic signaling networks[1].

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Background

Eukaryotes coordinate metabolism through a conserved signaling hub, the TARGET OF RAPAMYCIN (TOR) atypical serine/threonine kinase[2–9]. Nutrients and some hormones stimulate TOR, which phosphorylates substrate proteins to engage multiple downstream pathways that broadly promote growth and anabolism[10–13]. When nutrients are limiting or conditions are unfavorable for growth, TOR activity declines and cells become quiescent[14]. TOR dysregulation causes or contributes to diverse human diseases, including cancers and age-related disorders, which has provoked significant investigation of TOR signaling networks in biomedical models[15]. These efforts have only recently started to reveal how TOR can decipher myriad upstream cues to modulate precise downstream responses. Much less is known about the TOR signaling network in plants, but plant biologists are increasingly interested in the potential benefits of genetically leveraging TOR regulatory systems to create resilient, high-yielding crops for a sustainable agricultural future.

Plants continually grow during their vegetative life cycle through cell division and expansion at shoot and root meristems and through cell expansion beyond the meristems[16]. Cell division and expansion are both developmentally coordinated by several phytohormones, including the gaseous phytohormone ethylene. Ethylene is popularly familiar for its role in fruit ripening: many fruits, such as apples and bananas, depend on ethylene for ripening. Ethylene also promotes seed germination, regulates development, and mediates responses to various abiotic and biotic stresses. At a molecular level, ethylene engages a well-defined signal transduction cascade that was first dissected through forward genetic screens for ethylene-insensitive mutants of *Arabidopsis thaliana* (Figure 1)[17–19]. One of the ethylene signaling components, ETHYLENE-INSENSITIVE 2 (EIN2), is a signaling hub of elusive molecular function. EIN2 appears to play multifaceted roles in plant cells, since several *ein2* alleles have been found in forward genetic screens for responses to various signals, including glucose[20], paraquat-triggered oxidative stress[21], and the phytohormones auxin[22], cytokinin[23], abscisic acid[24,25], and jasmonic acid[21]. Since ethylene responses are blocked in *ein2* mutants, these effects could reveal general connections between ethylene biosynthesis/signaling and other pathways (e.g., cytokinin acts, in part, by promoting ethylene biosynthesis), but several of these responses cannot be readily explained through the role of EIN2 in ethylene signaling. In their Landmark report, Fu *et al.*[1] make major advances in understanding how TOR and EIN2, two molecular signaling hubs, cooperate to coordinate plant responses to diverse upstream cues and regulate growth and development.

Main contributions and importance

When seedlings are grown in complete darkness, a situation analogous to germination under the soil, their hypocotyls (embryonic stems) elongate until the seedlings encounter light. Unlike many stages of plant development, hypocotyls elongate exclusively through cell expansion, not division[26–28]. TOR and ethylene antagonistically regulate hypocotyl elongation: ethylene causes dark-grown seedlings to form short, thick hypocotyls[19], whereas TOR promotes long, narrow hypocotyl growth[29]. To discover how TOR promotes hypocotyl elongation, Fu *et al.* screened for mutants involved in hypocotyl growth and discovered that mutants defective in ethylene responses, *ein2* and *ein3;ein3-like1*, are less sensitive to TOR inhibition and continue to grow even when TOR is inactivated. Surprisingly, however, *etr1* mutants defective in ethylene sensing upstream of EIN2 are not resistant to TOR inhibition, ethylene does not impact TOR activity in dark-grown seedlings, and inhibitors of ethylene biosynthesis and signaling also had no impact on TOR regulation of hypocotyl

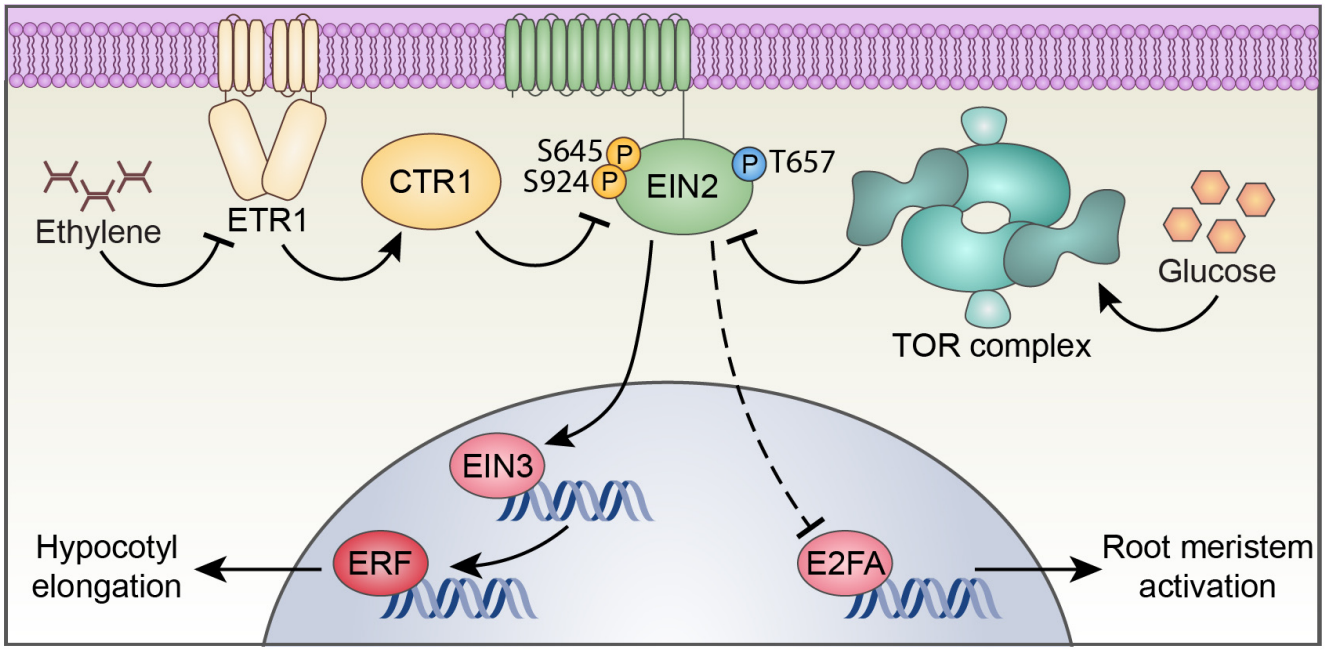

Figure 1. Ethylene-ETR1-CTR1 and glucose-TOR signaling converge on the EIN2 signaling hub

Ethylene is perceived at the ER membrane (purple) by a family of receptors that includes ETR1[30,31]. In the absence of ethylene, the receptors activate a cytosolic serine/threonine kinase, CTR1, which phosphorylates another ER-resident protein, EIN2, at residues Ser645 and Ser924[31]. Ethylene directly binds to and suppresses ETR1 receptors, preventing their activation of CTR1, and EIN2 (in the unphosphorylated state) is then proteolytically cleaved to release its cytosolic C-terminus from the ER membrane[32–34]; this fragment ("EIN2-C") promotes the expression and activity of EIN3 family transcription factors through multiple mechanisms in the cytosol and nucleus (blue)[35–39]. EIN3 transcription factors drive the ethylene-response transcriptional program, including by directly promoting transcription of the much larger ERF family of ethylene-response transcription factors[40–42]. A classical consequence of ethylene signaling is repressed hypocotyl elongation in the dark[19]. In this Landmark study, Fu *et al.* discover that the TOR complex phosphorylates another EIN2 residue, Thr657. They present evidence that full-length EIN2, which potentially has distinct functions from EIN2-C[21], accumulates in the nucleus when Thr657 is not phosphorylated by TOR[1]. When metabolic conditions are not favorable and TOR is inactive, Thr657 is not phosphorylated and EIN2 represses root meristem activity, at least in part by preventing E2FA-promoted cell cycle progression[1].

elongation. These results strongly suggested that TOR acts through an ethylene-independent pathway that converges on EIN2 to coordinate growth.

Fu *et al.* next deployed a series of elegant experiments to demonstrate that TOR directly interacts with EIN2 and phosphorylates Thr657, a broadly conserved residue in orthologues of EIN2 that had not been previously investigated. Biochemically mimicking EIN2-Thr657 phosphorylation in mutated "phosphomimetic" EIN2-T657D transgenic lines was sufficient to render seedlings insensitive to TOR inhibition, demonstrating that TOR-EIN2 signaling is critical to drive hypocotyl elongation. Strikingly, the phosphomimetic EIN2-T657D lines remain fully sensitive to ethylene, which prevents phosphorylation of two different EIN2 residues, Ser645 and Ser924. Oppositely, phosphomimetic EIN2-S645D lines are ethylene-insensitive but remain sensitive to TOR inhibitors. Therefore, TOR and ethylene signaling intersect at the EIN2 signaling hub but act on EIN2 through distinct phosphosites to regulate hypocotyl elongation. Moreover, transcriptional

analysis of light-grown wild-type and mutant seedlings revealed that EIN2 is required for a majority of the glucose-triggered, TOR-dependent responses, suggesting that EIN2 is a key effector of TOR metabolic programming in plants.

Open questions

This Landmark study provides a compelling model for understanding how cellular signal transduction networks interconnect and opens several new avenues for investigation in plant biology. Fu *et al.* provide evidence that TOR-catalyzed EIN2-Thr657 phosphorylation prevents translocation of full-length EIN2 from the ER to the nucleus, whereas ethylene promotes cleavage and release of a soluble C-terminal fragment of EIN2 (EIN2-C) that promotes ethylene responses in the nucleus and cytosol. This raises the possibility that full-length EIN2 has distinct activities from EIN2-C in the nucleus, and might constitute an uncharacterized translocation mechanism, since it is not obvious how a transmembrane ER protein could relocalize to the nucleus.

At the organismal level, both ethylene and TOR regulate growth, development, and physiology in contexts beyond the Arabidopsis seedling models used in this study. Does EIN2 mediate TOR signaling in these contexts? How do TOR and EIN2 interact to modulate responses to metabolic status, ethylene, and other phytohormones when plants experience abiotic stress, during ripening and senescence, or during pathogen attack? The discovery that TOR and EIN2 work closely together could help to illuminate how phytohormone signals and metabolic cues intersect throughout a plant's lifespan.

Evolutionarily, ethylene signaling arose in early algal ancestors of plants[43] and TOR was already present in the last eukaryotic common ancestor[9]. Moreover, the TOR-catalyzed phosphosite of EIN2, Thr657, appears to be conserved even in some bryophytes, hinting that TOR-EIN2 regulation may have evolved in the earliest land plants. Therefore, determining whether the TOR-EIN2 signaling hub is functionally conserved beyond the Arabidopsis model system could reveal new targets for agricultural scientists working to breed resilient, high-yielding crops.

Conclusion

The convergence of TOR and EIN2 signaling networks through direct molecular and functional interactions illustrates how complex upstream cues can be deciphered by cells to modulate specific downstream responses. This creative investigation from Fu *et al.* is a stellar example of how cell and molecular biology can be used to address classical problems—in this case, how plants integrate various signals, including nutrients and hormones, to coordinate growth—and reveal underlying mechanisms. Going forward, the discovery of the TOR-EIN2 signaling hub will serve as a model for investigations of cellular signal transduction and provoke new fundamental and translational research in plant physiology and development.

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