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Meta-regulation of *Arabidopsis* Auxin Responses Depends on tRNA Maturation

**Graphical Abstract**

**Highlights**

- *Arabidopsis* Elongator post-transcriptionally modulates PIN protein levels
- *Arabidopsis* tRNA splicing modulates auxin responses
- *Arabidopsis* tRNA maturation links basic cellular activities to developmental readouts

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**In Brief**

Leitner et al. analyze *Arabidopsis* lines deficient in distinct aspects of tRNA maturation, finding defects in control of auxin transport and signaling events. The occurrence of such auxin-related defects as a result of general deficiencies in translational control underlines a rate-limiting function for auxin in plant development.
Meta-regulation of Arabidopsis Auxin Responses Depends on tRNA Maturation

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SUMMARY

Polar transport of the phytohormone auxin throughout plants shapes morphogenesis and is subject to stringent and specific control. Here, we identify basic cellular activities connected to translational control of gene expression as sufficient to specify auxin-mediated development. Mutants in subunits of Arabidopsis Elongator, a protein complex modulating translational efficiency via maturation of tRNAs, exhibit defects in auxin-controlled developmental processes, associated with reduced abundance of PIN-formed (PIN) auxin transport proteins. Similar anomalies are observed upon interference with tRNA splicing by downregulation of RNA ligase (AtRNL), pointing to a general role of tRNA maturation in auxin signaling. Elongator Protein 6 (ELP6) and AtRNL expression patterns underline an involvement in adjusting PIN protein levels, whereas rescue of mutant defects by auxin indicates rate-limiting activities in auxin-controlled organogenesis. This emphasizes mechanisms in which auxin serves as a bottleneck for plant morphogenesis, translating common cellular activities into defined developmental readouts.

INTRODUCTION

Sessile plants evolved sophisticated mechanisms to respond to environmental stimuli and developmental cues. Key to several of these processes is the transmission of signals generated by the plant hormone auxin (indole-3-acetic acid, IAA, the predominant form found in plants). Auxin is special among plant hormones as its spectrum of activities requires its active, directional transport throughout the plant body (Peer et al., 2011; Voß et al., 2014; Wabnik et al., 2011). Such polar auxin transport (PAT), together with metabolic control of auxin, defines local variations in hormone levels, which are perceived and transmitted to induce hormonally controlled adjustments in gene expression and activity (Ljung, 2013; Sauer et al., 2013).

Perception of variations in auxin homeostasis and downstream signaling events has been analyzed extensively in the model plant Arabidopsis, which led to establishment of pathways specifically required for the transmission of hormonal signals (Boer et al., 2014; Dhamasiri et al., 2005; Kepinski and Leyser, 2005; Paciorek et al., 2005; Ulmasov et al., 1999; Xu et al., 2010). This involves cellular auxin uptake and efflux proteins with plasma membrane-localized PIN proteins implicated in auxin efflux (Petrásek et al., 2006). Here, we describe components of two distinct Arabidopsis pathways involved in tRNA maturation and characterize their function in auxin distribution and responses. We show that adjustments in PIN auxin transport protein levels are mediated by a plant ortholog of the multifunctional Elongator complex. In eukaryotes, Elongator has been linked to diverse cellular events, including transcriptional elongation, acetylation of chromatin and cytoskeleton components, protein sorting, as well as post-transcriptional modification of tRNAs (Chen et al., 2009; Esberg et al., 2006; Otero et al., 1999; Rahl et al., 2005; Wittschieben et al., 1999). We provide evidence that alterations in Elongator-mediated tRNA maturation in plants give rise to auxin-related growth deficiencies and PIN mis-expression. In addition, Arabidopsis deficient in AtRNL, an RNA-ligase required for splicing of tRNAs that do not represent bona fide ELP substrates, exhibits growth defects resembling those of elp mutants. This suggests a more common requirement of tRNA maturation for morphogenesis and auxin responses. Expression patterns of ELP6 and AtRNL, together with auxin-induced rescue of growth defects associated with diminished tRNA levels, are consistent with scenarios in which tRNA availability modulates auxin responses.

Overall, we demonstrate that deficiencies in Arabidopsis tRNA maturation and availability broadly concern transmission of auxin signals. This underlines rate-limiting roles of the growth regulator in plant morphogenesis and hints at meta-regulatory levels of growth control, by which adjustments in general cellular activities could feed back on defined aspects of plant development via modulation of auxin responses.
RESULTS

Mop Mutants Are Affected in Subunits of an Arabidopsis Elongator Complex

In earlier work, we described two Arabidopsis mutants termed mop2-1 and mop3-1 (for modulator of PIN), which both exhibit developmental and patterning defects reminiscent of mutants deficient in PAT (Malenica et al., 2007). This is indicated by altered expression of an auxin-responsive reporter protein and by reduced abundance of PIN auxin transport proteins. Notably, although PIN protein levels are reduced in both mop mutants, no corresponding changes in PIN transcript levels could be detected, suggesting involvement of both MOP loci in post-transcriptional regulation of PINs (Malenica et al., 2007).

Both mop mutants are derived from a pool of T-DNA insertion lines (Sieberer et al., 2003). For cloning of MOP2, we analyzed F2 populations of mop2-1, outcrossed into Col-0 wild-type, and identified a segregating T-DNA insertion in the second intron of locus At4g10090. This locus represents an Arabidopsis ortholog of Elongator protein 6 (ELP6), a subunit of Elongator holoenzyme found in eukaryotes from baker’s yeast to human (Hawkes et al., 2002; Nelissen et al., 2005; Otero et al., 1999). To test whether mop2-1 mutant phenotypes could result from this T-DNA insertion we analyzed gene expression, demonstrating a loss of ELP6 transcription in mop2-1 (Figure S1B). Transformation of mop2-1 with a VENUS-tagged ELP6 fusion protein expressed by its endogenous promoter (ELP6p::VENUS:ELP6) rescued growth deficiencies, indicating that mop2-1 defects result from a loss of ELP6 (Figures S1C and S1D). We therefore termed the former mop2-1 allele elp6mop2.

The Elongator complex was suggested to be composed of 12 subunits, with two copies of each ELPI, 2, and 3 forming a core sub-complex, and an additional accessory sub-complex, composed of two copies of ELPI, 5, and 6 (Glatt and Müller, 2013; Winkler et al., 2001). Given the resemblance of elp6mop2 and mop3-1 phenotypes, we reasoned that the latter could be affected in another ELP locus and initiated complementation tests between mop3-1 and T-DNA insertion lines affected in the remaining subunits of Arabidopsis Elongator. No complementation of mutant phenotypes was observed in F1 and F2 progeny derived from crosses between mop3-1 and GK_555H06, deficient in ELPI (At5g50320; ELONGATA 3, HISTONE ACETYLTRANSFERASE OF THE GNAT FAMILY 3). Determination of ELP3 expression in mop3-1 demonstrated a loss of full-length transcript (Figure S1B); however, when analyzing the genomic ELP3 locus in mop3-1, we could not characterize a T-DNA insertion but found evidence for rearrangements at this chromosomal position (Leitner, 2011). Therefore, and to minimize potential effects unrelated to defects in ELP3, all further experiments were performed with line GK_555H06, harboring a characterized T-DNA insertion in the ELP3 locus (elp3; Xu et al., 2012; Figure S1B).

Arabidopsis Elongator Is Involved in Post-transcriptional Control of PIN Protein Levels

The phenotypic resemblance of elp6mop2, mop3-1, and elp3, suggests that corresponding gene products function as essential subunits of a protein complex, similar to Elongator in other eukaryotes (Glatt and Müller, 2013). We therefore tested mutants defective in the remaining ELP subunits for growth defects (Figure S1B). Homozygous insertion lines for ELP1/ELO2/ABO1 (At5g13680; SALK_005153), ELP2 (At1g49540; SALK_106485), ELP4/ELO1 (At3g11220; SALK_079193), and ELP5 (At2g18410; GK_700A12) were grown along elp3 and elp6mop2. All these mutants exhibited related defects in shoot and root morphology, manifested at the seedling stage as reduced root elongation and deficiencies in directional root growth along the gravity vector, similar to mop alleles (Figures 1A–1C; Malenica et al., 2007). Related developmental defects were observed during later stages of development, affecting leaf and inflorescence morphogenesis (Figure S1A).

Elp phenotypes in patterning and growth responses are reminiscent of mutants in auxin transport and/or signaling (Malenica et al., 2007). In accordance, a comparison of endogenous PIN1 and PIN2 protein levels in all six elp mutants demonstrated reduced abundance of these key regulators of auxin transport, and consistent results were obtained when analyzing expression of the PIN1p::PIN1:GFP translational reporter gene (Figures 1D and 1E). Defects in protein abundance are not restricted to PINs though, exemplified by reduced accumulation of a BOR1:GFP translational fusion, when overexpressed by the 35S promoter in elp6mop2 (Figure S2C; Takano et al., 2005).

We analyzed subcellular distribution of PIN1 in further detail and tested for endogenous PIN1 in soluble elp protein fractions and determined PIN1:GFP localization at higher resolution. No striking differences to wild-type could be observed in these experiments, suggesting that reduced PIN abundance in the membrane fraction is not an immediate consequence of protein mis-localization (Figures S2A and S2B). Evidence for elp deficiencies affecting post-transcriptional regulation of PINs came from analysis of PIN transcript levels in the elp mutants. Specifically, no decrease in PIN1 transcript levels was detectable with qPCR, whereas protein levels were decreased (Figure 1D). A slight decrease in transcript levels was observed for PIN2 in elp mutants, which, however, was significant only for elp2 (Figure 1D).

To test for crosstalk between Elongator and PINs, we generated PIN1 overexpression constructs under control of the RP40 ribosomal protein promoter, highly active in meristematic zones (Butt et al., 2014). We made use of this promoter, because attempts to obtain efficient PIN overexpression lines in mop mutants failed, when using the CaMV 3SS promoter (Malenica et al., 2007). Expression of RP40p::PIN1 in elp6mop2 resulted in markedly higher PIN1 protein levels, demonstrating that RP40p-driven PIN1 expression is well suited for this approach (Figure S3A). When analyzing phenotypes, we found that elp6mop2 RP40p::PIN1 primary roots are longer than elp6mop2 roots of identical age whereas no pronounced difference was observed when comparing wild-type and RP40p::PIN1, indicating that reduced abundance of PIN1 contributes to elp6mop2 root growth defects (Figure 2A). We then determined responses of elp6mop2 RP40p::PIN1 to auxin transport inhibitor NPA (1-N-Naphthylphthalamic acid), as elp6mop2 seedlings are overly sensitive to NPA (Malenica et al., 2007). NPA sensitivity is dampened in elp6mop2 RP40p::PIN1, suggestive of a PIN1 dosage-dependent reversion of NPA
effects on root elongation (Figure 2A). Consistent with this scenario, NPA-induced ectopic cell proliferation of elp6mop2 root tips is antagonized by PIN1 overexpression, illustrated by reduced ectopic formation of root cap cells (Figures 2B and 2C). However, at the stage of flowering, elp6mop2 RP40p::PIN1 still exhibits marked morphological defects (Figures S3B and S3C). Thus, while PIN1 overexpression is not adequate for a full reversion of elp6mop2 phenotypes, our observations establish a causal link between downregulation of PIN1 and elp6mop2 root growth defects.

ELP-Mediated tRNA Maturation Influences Auxin Responses in Arabidopsis

Arabidopsis Elongator has been associated with diverse processes, revealing widespread roles for the protein complex (Nelissen et al., 2005; Wang et al., 2013b; Xu et al., 2012; Zhou et al., 2009), and phenotypes of the elp mutants point toward a distinctive role in transmitting auxin signals, which earlier has been linked to control of transcriptional elongation (Nelissen et al., 2010). In addition, and analogous to Elongator in yeast, a mutation in Arabidopsis ELP3 was found to be defective in uridine modification of tRNAs, essential for wobble codon recognition during protein translation (Mehlgarten et al., 2010). We analyzed tRNA preparations from elp6mop2 and elp3 and observed a reduction in the occurrence of 5-methoxycarbonyl-methyl-2-thiouridine (mc3′s2U) and of 5-carbamoyl-methyluridine (nc5U) in both mutant alleles (Figure 2D). This is in accordance with earlier observations and substantiates a role of Arabidopsis Elongator in tRNA maturation at wobble position 34, to improve wobbling accuracy and translational fidelity (Johansson et al., 2008; Mehlgarten et al., 2010).

Given that Elongator has been linked to several cellular activities, we asked whether explicitly deficiencies in tRNA maturation could contribute to Elongator mutant phenotypes in...
Arabidopsis. To this end, we made use of Kluyveromyces lactis γ-toxin, an endonuclease preferentially degrading tRNAs that underwent modification by Elongator (Lu et al., 2005). Assuming that reduced abundance of Elongator-modified tRNAs contributes to elp mutant phenotypes, γ-toxin-mediated degradation of such tRNAs would be expected to give rise to related growth defects. Expression of γ-toxin (gam) under control of the RP40 promoter (Figure 3A) resulted in auxin-related defects, resembling those of elp mutants (13/20 lines analyzed). This involved defects in root growth, cotyledon formation (18/30, 0/30 in controls) and aberrations in lateral organ positioning at inflorescence axes (25/62, 4/50 in controls; Figures 3C–3F). Moreover, PIN1:GFP reporter signals were reduced in RP40p::gam seedlings, while PIN1 transcript levels appeared unaffected, indicating that ectopic gam expression interferes with post-transcriptional control of PIN1 (Figures 3G–3I). To test whether RP40p::gam is affected in tRNA abundance, we determined steady-state levels of potential γ-toxin substrates, namely, tRNAs with a uridine at wobble position 34. In these experiments we normalized amounts of tRNAGlnUUG and tRNAGluUUC to levels of tRNAHisGUG or tRNASerAGA, the latter two lacking a uridine at the wobble position, serving as internal standards (Figure 3B). We detected reduced abundance of tRNAGlnUUG and tRNAGluUUC in RP40p::gam lines when compared to wild-type, suggesting that γ-toxin is active in Arabidopsis with substrate specificities similar to those observed in yeast.

Next, we tested for genetic interaction between Elongator and RP40p::gam by crossing RP40p::gam into elp6mop2 followed by analysis of the resulting progeny. Double homozygous elp6mop2 RP40p::gam strongly resembled parental RP40p::gam and elp6mop2 lines, which would be consistent with overlapping tRNA substrate specificities of Elongator and γ-toxin, when expressed in Arabidopsis (Figures S4A–S4F). However, elp6mop2 RP40p::gam appeared delayed in their development, when compared to parental lines (Figure S4G), indicating synergistic interaction between γ-toxin and Elongator, which points to an incomplete overlap of activities of these genetic traits.

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35S::YFP:GCN5, overexpressing a bona fide histone acetyltransferase, exclusively exhibited nuclear signals under our experimental conditions (compare Figures 4D–4F; Earley et al., 2007). Thus, analogous to observations made for Elongator in S. cerevisiae, cytoplasmic signals exhibited by Arabidopsis ELP reporter proteins are in agreement with a role of Elongator outside the nucleus and could involve a function in tRNA maturation (Huang et al., 2005; Rahl et al., 2005).

**tRNA Processing as a General Determinant of Auxin Responses in Plants**

Analysis of elp mutants and RP40p::gam point to a requirement of tRNA maturation for correct auxin responses. We asked whether this effect could be specific for tRNAs modified by Elongator or represents a more general response to alterations in tRNA homeostasis. We therefore analyzed Arabidopsis RNA LIGASE (AtRNL), a locus resembling yeast RNA ligase Trl1, implicated in splicing of tRNAs (Englert and Beier, 2005; Wang et al., 2006). In Arabidopsis, this would predominantly affect maturation of tRNA^{Tyr}, composed of a gene family of 76 loci, out of which 70 are predicted to contain an intron, whereas only a smaller sub-fraction of tRNA^{Met} (11/24) and tRNA^{Ser} (2/64) loci contains an intron (http://lowelab.ucsc.edu/GtRNAdb/Athal/). However, none of these tRNAs represents a likely target for Elongator, as they lack a uridine at their wobble position.

First, we analyzed expression of AtRNLp::GUS reporter transformed into Arabidopsis and found intense GUS staining in root meristems and in the vasculature of developing plantlets as well as in flowers and elongating tissue, pointing to a requirement for tRNA splicing preferentially in proliferating cell files and tissues (Figures S5A–S5G). Next, we sought for insertion mutants but
failed to obtain lines with noticeable effects on AtRNL gene expression (see the Supplemental Information). We therefore took another approach and generated artificial microRNA lines (Schwab et al., 2006), in which transcription of endogenous AtRNL was downregulated (Figure 5A; Figures S6A and S6B). When determining splicing of tRNA_{Tyr} in RP40p::amiRNL-b seedlings at 5 days after germination (DAG), we detected a strong reduction in the amounts of processed tRNA, suggesting that these plants contain only limited quantities of functional tRNA_{Tyr} due to deficiencies in the splicing reaction (Figure 5B).

Figure 4. Expression Analysis of ELP Reporter Genes  
(A and B) ELPl6p::VENUS:ELP6 expression (yellow) in developing true leaf (A) and primary root meristem (B) at 6 DAG.  
(C) Cytoplasmic distribution of ELP6p::VENUS:ELP6 in root meristem stele cells. Areas with reduced signal intensities represent nuclei. Seedlings were stained with PI (red) to visualize cell and organ boundaries.  
(D) Expression of 35S::YFP:ELP3 (yellow signals) in 6-day-old Col-0 root meristem cells results in cytoplasmic and additional nuclear signals. To visualize nuclei, seedlings were fixed and counterstained with DAPI (blue).  
(E) Expression of 35S::YFP:GCN5, encoding a nuclear-localized protein, in Col-0 root meristem cells under conditions identical to those in (D). Size bars represent 100 μm (A), 50 μm (B, D, and F), and 10 μm (C and E).

Morphology and development of RP40p::amiRNL-b resembled those of elp1/mop mutants (10/16 lines analyzed). Specifically, RP40p::amiRNL-b seedlings exhibited reduced root elongation, aberrations in cotyledon number (49/91; 1/35 in Col-0) and incomplete cotyledon venation (17/40; 0/35 in Col-0) as well as deficiencies in root meristem morphology and root gravitropism (Figures 5C–5E, 5J, and 5K). At later developmental stages, we observed reduced apical dominance and aberrations in lateral organ positioning at inflorescence stems (27/62; 2/38 in Col-0; Figures 5H and 5I). Related growth defects have been linked to alterations in auxin responses, and we therefore analyzed expression of auxin responsive DR5rev::GFP (Benková et al., 2003). Reporter signals in RP40p::amiRNL-b DR5rev::GFP primary root meristems appeared reduced (Figures 5J and 5K), indicating alterations in auxin distribution and/or signaling that coincide with deficiencies in tRNA splicing.

To validate our observations, we generated another amiRNL construct, expressed under control of the 35S promoter, and targeting a different region of the AtRNL mRNA. Transgenic 35S::amiRNL-a seedlings with reduced abundance of AtRNL transcript exhibited phenotypes resembling those of RP40p::amiRNL-b (Figures S6B–S6F). This corroborates a scenario in which phenotypes observed result from downregulation of AtRNL and associated aberrations in tRNA splicing. Moreover, the resemblance of amiRNL and elp loss-of-function mutants, both predicted to impact on distinct sets of tRNA families, indicates that non-overlapping limitations in tRNA availability give rise to comparable developmental defects.

tRNA Maturation Acts in the Transmission of Instructive Auxin Cues

To further define the role of Elongator and AtRNL in auxin-controlled morphogenesis, we analyzed lateral root formation, a process that depends on coordinated distribution of auxin (Benková et al., 2003). When viewing AtRNL::GUS activity, GUS staining became visible already in stage I lateral root primordia, and strong expression remained detectable during later stages of organ development (Figures 6A and 6B). Likewise, in elp6^{mop2} ELPl6p::VENUS:ELP6 signals became detectable upon primordium development and remained visible during further growth (Figures 6C and 6D). This points to a function for Elongator and AtRNL in lateral root growth, and we next determined expression of PIN1p::PIN1:GFP in lateral roots of wild-type, elp6^{mop2}, and RP40p::amiRNL-b seedlings. PIN1::GFP signals in elp6^{mop2} turned out to be weaker than in wild-type, and similar findings were made, when analyzing reporter signals in RP40p::amiRNL-b lateral roots (compare Figures 6E–6J). Thus, as observed for primary root meristems (Figure 1; Figures S6G–S6I), PIN1 expression appears reduced in lateral roots upon interference with tRNA maturation.

We then tested for possible consequences of reduced PIN expression and compared lateral root growth in wild-type and elp6^{mop2}. This revealed a reduction in the number of elp6^{mop2} lateral roots, when compared to wild-type (Figure 6K). When analyzing RP40p::amiRNL-b, we observed a reduction in lateral root formation as well, but effects in the silencer lines appeared less pronounced and more variable than in elp6^{mop2} (Figure 6K). When performing root growth experiments in the presence of low concentrations of the auxin analog 1-naphthalene acetic acid (NAA) without noticeable effects on lateral root growth in wild-type, we detected rescue of lateral root formation specifically in elp6^{mop2} (Figure 6K). This is in accordance with limitations in auxin availability and/or distribution causing lateral root growth deficiencies associated with the mutant, and consistent with a role of Elongator in transmission of instructive auxin signals during root morphogenesis. Taken together, our findings link tRNA maturation to root organogenesis, potentially via mechanisms...
orchestrating PIN activities and associated adjustments in auxin distribution.

**DISCUSSION**

Recent years have seen characterization of a range of signaling pathways in higher plants, revealing molecular switches that communicate signals essential for plant development. Auxin appears to be transmitted by distinct pathways, with SCFTIR1/AFB-dependent hormone perception predominantly affecting transcriptional control (Dharmasiri et al., 2005; Kepinski and Leyser, 2005). Another pathway, suggested to modulate protein sorting in response to auxin, seemingly requires ROP-GTPase activity (Paciorek et al., 2005; Xu et al., 2010), while involvement of putative auxin receptor auxin binding protein 1 in auxin-controlled development has been seriously challenged very recently (Gao et al., 2015). The identification of components acting in auxin signaling provided insights into principles that might confer specificity to such hormonal responses, but additional reports demonstrated that less specific cellular processes contribute to the orchestration of auxin effects as well (Dhonukshe et al., 2008; Kitakura et al., 2011; Korbei et al., 2013; Rosado et al., 2010, 2012; Wang et al., 2013a). Our findings establish tRNA maturation as a highly general effector of auxin responses, acting via translational control of PIN proteins, and provoking questions on function and specificity of such activities in the regulation of hormonal signaling (Retzer et al., 2014).

**Figure 5. Analysis of AtRNL RNA Ligase Knockdown Lines**

(A) qPCR displaying AtRNL transcript levels in RP40p:amiRNL-b lines 2–8 and 6–8 at 6 DAG (Col-0 = 1). Results from three biological repeats with three technical replicates are shown. SDs are indicated (*Student’s two-tailed t test, p < 0.01).

(B) Quantification of tRNA_{Tyr}^{GUA} levels in Col-0 and RP40p:amiRNL-b silencer lines by PCR. Upper band corresponds to non-spliced tRNA_{Tyr}^{GUA} precursor (black arrowhead); lower band corresponds to processed tRNA_{Tyr}^{GUA} (red arrowhead). Ratios of spliced and precursor band intensities are displayed below.

(C and D) Col-0 (C) and RP40p:amiRNL-b (D) seedlings at 6 DAG. White arrowhead in (D) points to fused cotyledons.

(E) Orientation of primary root growth of Col-0 and RP40p:amiRNL-b lines 2–8 and 6–8 seedlings at 8 DAG. A total of 40–50 seedlings was analyzed for each genotype and plotted as percentage of seedlings displaying <15°, <30°, <45°, and >45° deviation from the vertical growth axes.

(F and G) Cotyledon vasculature in Col-0 (F) and RP40p:amiRNL-b (G). White arrowhead indicates vasculature discontinuity not observed in wild-type.

(H and I) Detail of inflorescence axes of flowering Col-0 (H) and RP40p:amiRNL-b (I) at 28 DAG. White arrowheads indicate fused, fasciated stem.

(J and K) Comparison of wild-type (J) and RP40p:amiRNL-b (K) primary root meristems expressing auxin-responsive DR5rev::GFP (yellow). Roots were stained with PI (red) to visualize cell boundaries. White arrowheads point to alterations in root meristem patterning.

Size bars represent 10 mm (C, D, H, and I), 1 mm (F and G), and 50 µm (J and K). See also Figures S5 and S6.
of two individual tRNAs can bypass major elf mutants phenotypes, presumably via compensating for the loss of translational fidelity that results from defects in Elongator (Esberg et al., 2006). Another link between Elongator and tRNA modifications came from crystallization of the ELP4/5/6 sub-complex (Glatt et al., 2012; Lin et al., 2012). These studies revealed a hexameric ring-shaped structure capable of interacting with the tRNA anticodon loop domain, which presumably represents a topological prerequisite for ELP-mediated ribonucleotide modification (Glatt et al., 2012). Moreover, analysis of protein abundance in an Schizosaccharomyces pombe Jelp3 mutant linked codon usage and Elongator-dependent variations in translational efficiency to discrete growth defects (Bauer et al., 2012). From all that, it seems that tRNA modification represents a major enzymatic function of Elongator (Glatt and Müller, 2013).

Like its fungal counterpart, Arabidopsis Elongator appears to function in diverse processes, including stress response, pathogen defense, and hormonal signaling, which has been attributed to Elongator’s activities in chromatin modification and transcriptional control (Chen et al., 2006; Nelissen et al., 2010; Sanmartín et al., 2011; Wang et al., 2013b; Zhou et al., 2009). Our results now establish links between Elongator-mediated tRNA modification and auxin-controlled pattern formation. Apart from reduced uridine modifications in tRNA preparations of elf/mop mutants, phenotypes observed upon expression of γ-toxin in Arabidopsis further substantiate such a role. Experiments in baker’s yeast demonstrated that tRNAs featuring mcm5s3Um or mcm7U at their wobble position represent preferred targets for γ-toxin (Lu et al., 2005), and our results point to related γ-toxin substrate specificities in Arabidopsis. In accordance, we observed comparable phenotypes when analyzing elf/mop mutants and RP40p::gam lines, which is consistent with overlapping tRNA substrate specificities of Arabidopsis Elongator and γ-toxin. Notably, although displaying phenotypes as their parental lines, growth of elf6mop2 RP40p::gam plants turned out to be retarded, when compared to its parents. Such additive phenotypes argue for an incomplete overlap in the activity of Arabidopsis ELP6 and γ-toxin. Alternatively, remaining tRNA modification activities in either elf6mop2 or RP40p::gam could be reduced further in elf6mop2 RP40p::gam plants. Whether these synergistic phenotypes reflect diverging activities in tRNA substrate processing or activities even unrelated to tRNA modifications remains to be addressed.

One might speculate about a function of Elongator in translational control, primarily required for the transmission of auxin signals. Limited PIN abundance in Elongator mutants, together with the finding that efficient overexpression of PIN1 partially rescues elf6mop2 growth defects, is in fact consistent with this model. Analysis of ATRNL, however, argues against such a scenario but rather implies less specific effects of tRNA availability on auxin-controlled growth and differentiation processes. The Arabidopsis tRNAγUA family likely represents a major substrate for the ATRNL RNA-ligase, indicated by pronounced deficiencies in tRNAγY splicing in amiRNL lines. In addition, members of the tRNAγCAU and tRNAγGU families represent potential ATRNL substrates, but none of these tRNAs is a likely target of Elongator, as they all lack uridine at wobble position 34. While this implies differing tRNA substrate specificities for Elongator and ATRNL, loss of either ELP or ATRNL still causes similar phenotypes, which points to a requirement of these distinct regulators of translational control in overlapping aspects of plant development.
Broad deficiencies in tRNA biogenesis as apparent in elp/mop and amiRNL should result in major changes in translational control, with reduced abundance of PINs representing only a small fraction of aberrations in protein translation. This would be in agreement with the various functions in signaling and stress responses attributed to Arabidopsis Elongator. In light of such diverse activities, it seems remarkable that several developmental defects associated with elp/mop mutants could be explained essentially by aberrations in auxin-controlled morphogenesis. Another report, linking tRNA function to auxin, described identification of an auxin response mutant, which turned out to be a member of the tRNA_{CGG} family (Perry et al., 2005). This mutant is predicted to erroneously and stochastically incorporate valine instead of alanine into growing peptide chains, nonetheless resulting in discrete defects in mediating auxin effects on cell proliferation (Perry et al., 2005). It remains to be determined how altered decoding characteristics of a single tRNA could give rise to such defined growth defects, which is all the more surprising as the Arabidopsis genome is predicted to encode 630 tRNAs, strongly suggestive of extensive functional redundancy (http://lowelab.ucsc.edu/GtRNAdb/Athal/).

The importance of tRNA maturation specifically for auxin responses and cell proliferation is underlined by our study, indicative of a high demand for ATRNL and Elongator activities in metabolically active and proliferating tissue. Limitations in the availability of tRNAs, as is the case for Elongator loss-of-function or ATRNL knockdown alleles, would translate into variations in the abundance of various proteins, including key mediators of plant development and pattern formation. PIN proteins seemingly represent such targets, as interference with tRNA maturation impacts on PIN levels, coinciding with modifications in auxin distribution and developmental reprogramming, which is reflected in major adjustments in proliferative growth. Rescue of elp6/mop2 lateral root growth defects by auxin supports such a scenario, as it indicates that aberrations in auxin availability or distribution are indeed accountable for elp/mop growth defects.

A plausible explanation for our observations implies that components of the auxin transport and/or signaling machinery represent rate-limiting determinants for plant morphogenesis. This is underlined by rescue of elp/mop root phenotypes via efficient PIN1 overexpression, likely compensating for suboptimal translation as a result of limiting availability of fully processed tRNAs. Related models have been put forward when studying tRNA availability of tRNAs, as is the case for Elongator loss-of-function. In light of such developmental aspects of cellular activity, including cytoskeleton function, endocytic protein sorting, and ribosome biogenesis, perturbation of which consistently resulted in phenotypes that could be linked to altered auxin responses (Dhonoukse et al., 2008; Kitakura et al., 2011; Retzer et al., 2014; Rosado et al., 2010; Wang et al., 2013a; Zhou et al., 2010). While none of these processes represents an integral element of auxin signaling pathways, it appears that plant cells have evolved mechanisms, conferring signaling specificity by means of highly general regulatory events. ROP-GTPases modulate intracellular sorting of PIN plasma membrane proteins and hence distribution of auxin (Xu et al., 2010). Moreover, activity of ribosome-associated proteins appears indispensable for translational re-initiation of ARF mRNAs, which contain regulatory uORFs, upstream of their actual Start-ATG (Rosado et al., 2012). However, such translational re-initiation is not restricted to transcriptional regulators of auxin responses, as about one-third of Arabidopsis protein-coding mRNAs is predicted to contain regulatory uORFs (von Arnim et al., 2014). Likewise, ROP activity is assumed to exert general effects on plasma membrane protein sorting not limited to auxin transport components, underpinning the role of PINs as well as additional elements of the auxin signaling machinery as a limiting bottleneck for plant development.

Insights into the mechanisms controlling tRNA maturation in higher plants are just emerging, which should contribute to our understanding of their function in plant development (Chen et al., 2010). Adjustments in tRNA maturation could, for example, arise as a consequence of variations in environmental parameters, causing modifications in translational control. In this scenario, reversible changes in the abundance of PIN auxin transport proteins resulting from fluctuations in translational efficiency would link fundamental cellular activities to defined events in pattern formation and morphogenesis. This would allow for efficient and rapid transmission of morphogenetic cues, simply by exploiting the rate-limiting role of auxin signaling for plant development. Experiments addressing environmental parameters and its effects on translational control are required to further test this hypothesis.

EXPERIMENTAL PROCEDURES

Plant Growth and Lines

Seedlings were cultured and analyzed as described (Korbei et al., 2013), in a 16-hr-light/8-hr-dark regimen at 22°C, unless indicated differently.

Generation of Constructs and Expression Analysis

Construct cloning and plant transformation was performed according to established protocols (Butt et al., 2014). Sample preparation and expression analysis has been described elsewhere (Butt et al., 2014; Korbei et al., 2013).

Microscopy

Seedlings were analyzed on Leica confocal laser scanning microscopes (CLSMs) as described (Korbei et al., 2013). A detailed description of all experimental procedures is provided as Supplemental Information.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.03.054.

AUTHOR CONTRIBUTIONS


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