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Short Title: RSL4-centered feedback loops shape root hair morphology

SHORT COMMUNICATION

RSL4-centered feedback loops shape root hair morphology

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Abstract

Root hairs significantly expand the surface area of roots in contact with the soil, thereby promoting water and nutrient uptake. Root Hair Defective 6-Like 4 (RSL4) is a key transcription factor regulating root hair development, yet the mechanisms underlying its post-translational regulation remain incompletely understood. Recently, we demonstrated that Calcium-dependent Protein Kinases (CPKs) integrate hormonal signals to phosphorylate RSL4, thereby enhancing its transcriptional activity. This Brief Report summarizes recent evidence on how RSL4 integrates environmental and hormonal cues at multiple regulatory levels to promote root hair growth.

Keywords: Root hairs, Root Hair Defective 6-Like 4 (RSL4), Feedback Regulation, Transcription, Translation, Transcription factor

Abbreviations: RSL4: Root Hair Defective 6-Like 4; LRH: Long Root Hair; RALF1: Rapid Alkalinization Factor 1; BDR6/7: Boundary of ROP Domain 6/7; CPK4/11: Calcium-Dependent Protein Kinase 4/11; RHS: Root Hair Specific; eIF4Es: Eukaryotic Translation Initiation Factor 4Es; FER: Feronia; RHE: Root Hair-Specific Cis-Element.

Introduction

Root hair function and plasticity

In vascular plants, root hairs are tubular outgrowths of root epidermal cells that dramatically expand the soil-root interface, facilitating the efficient absorption of water and nutrients for plant viability and mediating symbiotic associations with soil microbes (Gifford, et al. 2020, Lopez, et al. 2024). Given the considerable spatial and temporal heterogeneity of soil nutrient bioavailability, plants dynamically adjust root hair density and length to optimize nutrient uptake. Consequently, root hair morphology serves as an important indicator of prevailing soil conditions (Gifford, et al. 2020). studies underscore the pivotal role of RSL4.

RSL4 and root hair development

RSL4 and its homologs function as master transcription factors that regulate root hair development. By integrating upstream environmental and hormonal signals, they bind the Root Hair-specific cis-Element (RHE) to regulate a suite of Root Hair Specific (*RHS*) genes, thereby shaping root hair morphology (Marzol, et al. 2017). This regulatory mechanism is conserved not only in *Arabidopsis* but also throughout the tracheophyte (vascular plant) lineage (Hwang, et al. 2017). Moreover, RSL homologs in the moss *Physcomitrella* patens regulate protonemal development (Pires, et al. 2013). Collectively, these studies underscore the pivotal role of RSL4.

Feedback regulation

Accumulating evidence indicates that RSL4 is modulated by multiple downstream effectors, thereby fine-tuning root hair development (Fig.1). At the translational level, Long Root Hair (LRH) and Rapid Alkalinization Factor 1 (RALF1), both directly regulated by RSL4, affect the translation of *RSL4* mRNA (Zhu, et al. 2020, Cui, et al. 2024). LRH, which acts as a negative regulator of root hair growth, inhibits the association of eukaryotic translation initiation factor 4Es (eIF4Es) with *RSL4* mRNA, thereby suppressing RSL4 translation and root hair elongation (Cui, et al. 2024). In contrast, RALF1 promotes Feronia-mediated phosphorylation of eIF4E1, thereby enhancing the affinity of eIF4E1 for RSL4 mRNA and stimulating RSL4 translation (Zhu, et al. 2020). Interestingly, *RALF1* is the only gene reported to date to be transcriptionally repressed by RSL4 (Zhu, et al. 2020). At the post-translational level, Boundary of ROP Domain 6/7 (BDR6/7) and Calcium-Dependent Protein Kinase 4/11 (CPK4/11), both activated by RSL4, regulate RSL4 transcriptional activity (Li, et al. 2026, Xu, et al. 2026). BDR6/7 promotes root hair initiation and elongation by interacting with RSL4 and facilitating RSL4 binding to RHE-containing promoters (Xu, et al. 2026), whereas CPK4/11 phosphorylates RSL4, thereby enhancing its transcriptional activity (Li, et al. 2026). In addition, RSL4 has been reported to indirectly influence the expression of GLABRA2 (*GL2*) a root hair repressor (Qiu, et al. 2021), which in turn affects RSL4 expression through transcription factors such as RHD6 (Lopez, et al. 2024).

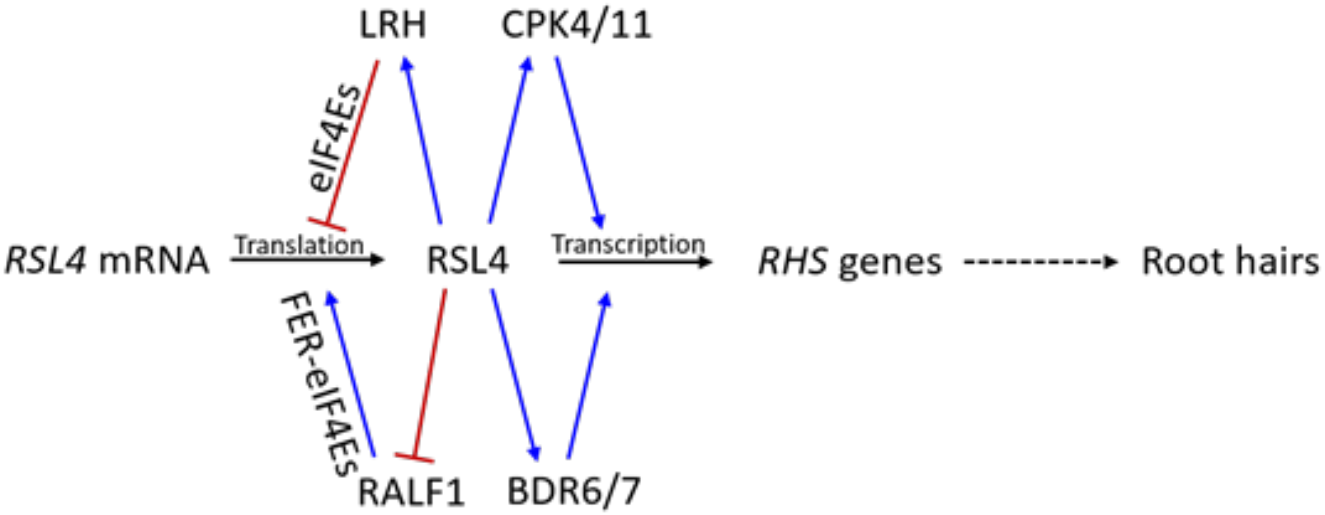

Figure 1. A model of RSL4-centered feedback loops shape root hair morphology. Note: RSL4: Root Hair Defective 6-Like 4; LRH: Long Root Hair; RALF1: Rapid Alkalinization Factor 1; BDR6/7: Boundary of ROP Domain 6/7; CPK4/11: Calcium-Dependent Protein Kinase 4/11; RHS: Root Hair Specific; eIF4Es: Eukaryotic Translation Initiation Factor 4Es; FER: Feronia; RHE: Root Hair-Specific Cis-Element. Blue lines indicate promotion, whereas red lines indicate inhibition.

Discussion

Collectively, these findings suggest that RSL4 frequently engages in feedback regulation with its downstream effectors, including LRH, RALF1 BDR6/7 and CPKs, thereby contributing to the spatiotemporal regulation of RSL4 throughout root hair development and ultimately shaping root hair morphology (Fig. 1). In light of the evolutionary conservation of RSL function (Pires, et al. 2013, Hwang, et al. 2017), it is plausible that similar feedback regulatory circuits operate in other plant species. Nevertheless, several outstanding questions remain: Which effectors within the root hair developmental signaling cascade modulate *RSL4* at the transcriptional level these feedback cascades act synergistically in response to fluctuating environmental cues.

Conclusion

These issues represent important avenues for future research? translational level, *RSL4* activates LRH transcription, and LRH subsequently suppresses *RSL4* mRNA translation *via* eIF4Es. Conversely, RSL4 represses RALF1 transcription, whereas RALF1 enhances *RSL4* mRNA translation through FER-dependent signaling. At the post-translational level, *RSL4* induces the transcription of CPK4/11 and BDR6/7, which in turn reinforce RSL4-mediated activation of *RHS* genes, thereby establishing positive feedback loops.

Author Contributions

Lu-Shen Li: Writing-original draft; Writing-review and editing.

Competing Interests

The authors declare no conflict of interest.

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