Trends in Plant Science

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Spotlight

RSL4 Takes Control: Multiple Signals, One Transcription Factor

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Root hair growth dramatically expands the root surface area, thus facilitating water and nutrient uptake. Until recently, the molecular mechanism underlying root hair growth was unknown. Recent studies have revealed that the transcription factor ROOT HAIR DEFECTIVE 6 LIKE 4 (RSL4) coordinates hormonal, environmental, and developmental factors to trigger polar growth.

One of the most intriguing questions in modern biology is how cells regulate their size. The rate at which cells grow is determined by both cell intrinsic factors and external environment signals. Root hairs are single plant cells that can expand to several hundred-fold their original size, and have emerged as an excellent model system for studying cell size regulation. Root hair development varies by plant species; it occurs either randomly, starting with an asymmetrical cell division, or via a position-dependent mechanism. The latter mechanism is better studied and occurs in the model plant *Arabidopsis thaliana,* where root hair cells, or trichoblasts, and nonhair cells, or atrichoblasts, differentiate from the epidermal cell layer. A well-defined developmental program and multiple environmental signals coupled to several hormones are integrated to define the final size of root hairs (Figure 1). Root hair size has vital physiological implications for the plant, determining the surface area: volume ratio of the all the roots exposed to the nutrient pools, thereby likely impacting nutrient uptake rates. Although the final hair size is of fundamental importance, the molecular mechanisms that control it remained largely unknown until recently. The developmental program, hormones, and environmental cues all converge to regulate the expression of the single basic helix-loop-helix (bHLH) transcription factor (TF) RSL4, which controls polar growth. Previous studies have discussed these three individual factors in detail [1–3]. Here, we discuss recent progress toward the elucidation of how the final size of root hair cells is fine-tuned by the master regulator, RSL4.

Specification of epidermal cell differentiation is a highly regulated process. A TF complex comprising WEREWOLF (WER), GLABRA 3 (GL3)/ENHANCER OF GLABRA 3 (EGL3), and TRANSPARENT TESTA GLABRA 1, induces the expression of GLABRA 2 (GL2), which inhibits root hair cell fate, suppressing ROOT HAIR DEFECTIVE 6 (RHD6) [4]. In trichoblasts, the lack of GL2 allows the expression of RDH6, leading to root hair initiation, a process that is controlled by the interplay between several genes, such as *RDH6–RSL1* [5]. Recently, it was shown that expression of *RSL4* under the control of the GL2 promoter, in both the wild-type and hairless *rhd6* mutant backgrounds, induced root hair growth in atrichoblasts. This suggests that *RSL4* expression induces root hair formation and growth independently of *RHD6–RSL1* [5]. This is likely because RSL4 regulates several genes involved in different key cell processes required for root hair growth (Figure 1).

Together with the developmental and genetic pathway, hormones, such as auxin (IAA for indole-3-acetic acid), ethylene (Et), cytokinin (CK), and strigolactones (SLs) are important triggers of root hair cell growth in trichoblasts [1–3]. Mechanistically, auxin needs to be sensed *in situ* in the root hair cells to trigger cell expansion (Figure 1). The slow transcriptional IAA response involves members of the TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX (TIR1/AFB) family and their co-receptor AUXIN/INDOLE 3-ACETIC ACID (Aux/IAA), and the concomitant release of AUXIN RESPONSE FACTORS (ARFs). ARFs bind to *cis*-auxin response elements (Aux-REs) in the promoters of early IAA response genes to trigger downstream responses [2]. Recently, it was shown that several ARFs directly upregulate RSL4 expression several-fold, linking IAA stimulation to RSL4 expression at the molecular level [6,7]. In addition, RSL4 was shown to promote reactive oxygen species (ROS) production by regulating the expression of two NADPH oxidases, C and J [also known as RESPIRATORY BURST OXIDASE HOMOLOG (RBOH) proteins] and several Class III apoplastic peroxidases (PERs) [5,7]. Chemical or genetic interference with ROS balance or peroxidase activity affects the final size of root hair cells. IAA stimulation of ROS production in root hair cells requires not only RSL4, but also RSL2, but how this hormonal program is coordinated remains to be determined [7]. Overall, these findings established a molecular link between IAA-regulated ARFs–RSL4 expression (and possibly RSL2) and ROS-mediated root hair growth [7]. In the case of Et, treatment with the Et precursor 1-aminocyclopropane-1-carboxylic acid (ACC) results in an enhanced root hair phenotype and the upregulation of several TFs, such as RHD6, RSL2, and RSL4, although the underlying molecular mechanism is obscure [3]. In addition, RSL4 contains one putative ARABIDOPSIS RESPONSE REGULATOR (ARR) type-B binding site in its regulatory sequence that possibly links *RSL4* with CK [3]; however, further studies are needed to confirm whether CK directly regulates *RSL4* expression. Finally, abscisic acid (ABA) was shown to repress root hair growth by inhibiting *RSL2* expression, but not that of *RSL4*, through the

activity of the OBF BINDING PROTEIN4 (OBP4) [8]. Several hormonal crosstalk and reciprocal regulation mechanisms exist in root hair cell sizing (e.g., IAA–Et signaling pathways can act in either an additive or synergistic manner), although the hierarchy of the signals is not fully understood.

Among environmental factors influencing root hair cell size, phosphate (Pi) availability in the soil is the best characterized [9,10]. In several plants, root hair growth is enhanced in low-Pi conditions and this adaptive response increases the capacity for Pi uptake. Exposure to Pi-deficient conditions immediately triggers *RSL4* expression, possibly via the PHOSPHATE STARVATION RESPONSE REGULATOR 1 (PHR1)/PHR1-like 1 (PHL1) TFs [9,10] (Figure 1). Gradients of other low-mobility mineral nutrients (e.g., iron and manganese) or rare elements (e.g., vanadium and boron) in the soil, as well as water status and levels of carbon monoxide, carbon dioxide, and catechol, a major chemical component of smoke, also affect root hair development, but the underlying molecular pathways are largely unknown. To make this picture even more complex, overlapping signaling pathways may exist between hormones, nutrients and the developmental program. For example, IAA, low Pi in the soil, and *RSL4* expression are all interlinked (Figure 1).

Recently, two novel RSL4 properties were uncovered. First, RSL4 is able to self-activate, enhancing its own expression [5]. Second, the rate of RSL4 synthesis determines the final size of the root hair cell and, because it contains a D-BOX motif, its protein stability is regulated by 26S proteasome degradation [10]. Accordingly, a mutated form of RSL4 that is stable and resistant to proteolysis develops abnormally long root hairs [10]. More importantly, RSL4 controls the expression of 124 genes (84 genes determined in [9], 29 in [11], and 11 in [12]) containing a root hair-specific *cis*-element (RHE) in

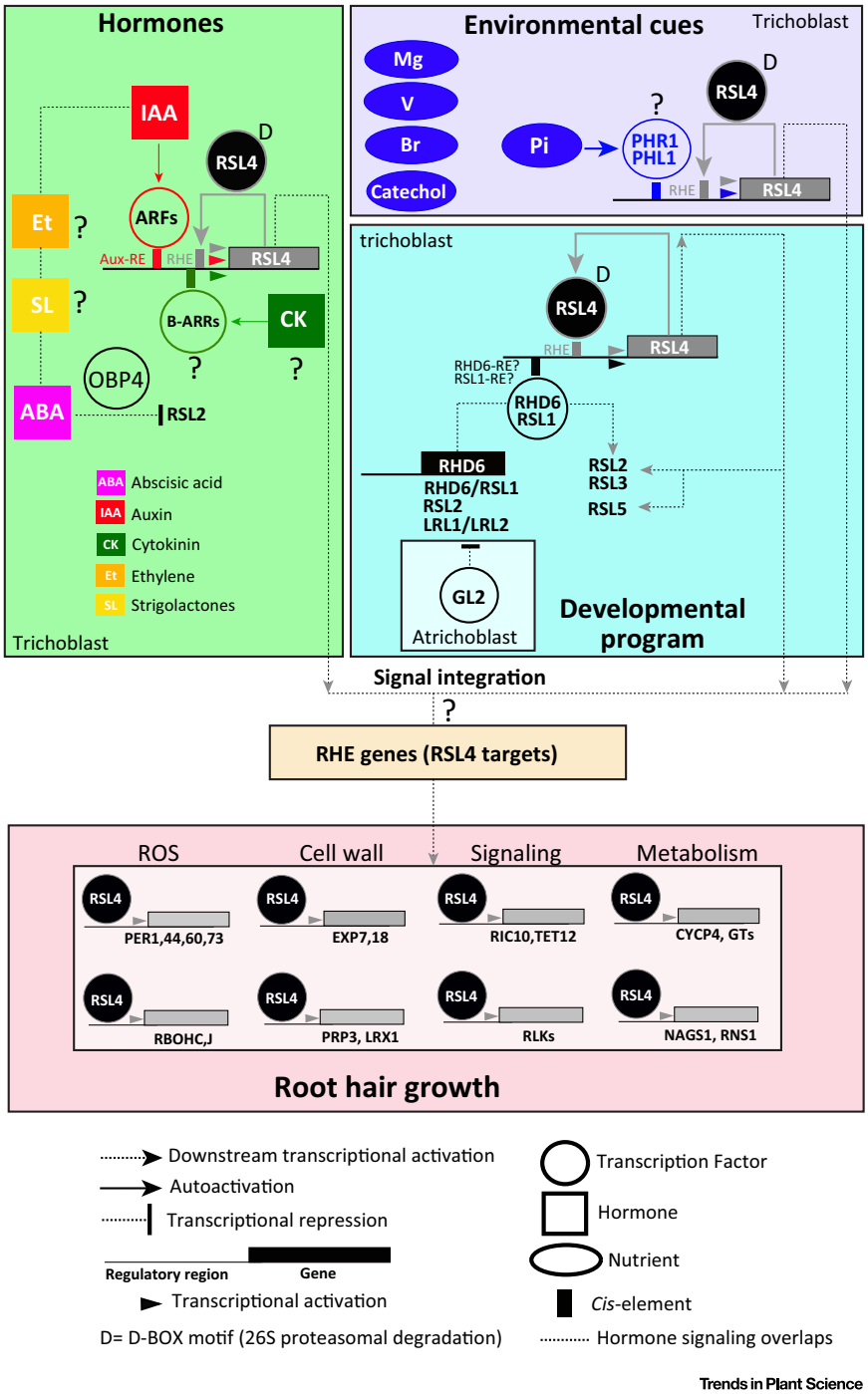

Figure 1. Multiple Signals Converge on the Key Regulator ROOT HAIR DEFECTIVE 6 LIKE 4 (RSL4) to Control Root Hair Cell Size. RSL4 is developmentally regulated by RHD6-RSL1 and controls several other RSLs (e.g., RSL2). GL2 is a main repressor of root hair development in atrichoblast cells (nonroot hair cells) that acts by inhibiting the expression of several transcription factors (TFs), including RHD6 and RSL4. Auxin is a key hormone that acts via multiple ARF activation to bind to a *RSL4* promoter on Aux-RE sites, upregulating its expression. Other hormones [e.g., ethylene (Et), cytokinin (CK), and strigolactones (SLs)] also influence root hair cell size, possibly by acting directly on RSL4 or on other TFs via unknown mechanisms. Environmental signals, such as low phosphate (Pi) also influence root hair cell size by triggering the activation of *RSL4* expression via PHR1-PHL1 TFs. Other external signals [e.g., low levels of nutrients, such as manganese (Mg), vanadium (V), and boron (Br), and catechol] also stimulate root hair cell growth. Since RSL4 contains a D-BOX motif in its

(Figure legend continued on the bottom of the next page.)

their regulatory regions [5]. These RSL4-activated genes function in ROS homeostasis, cell wall synthesis and remodeling, metabolism, and signaling, and represent the smallest subset of genes necessary to trigger root hair growth [5]. Together, these properties make RSL4 a key master regulator of final cell size that integrates environmental, hormonal, and developmental cues (Figure 1). Other TFs and transcriptional components not described here (e.g., LRL1-LRL3, mediator 25/PFT1, etc.) act in an RSL4-independent manner to regulate the expression of root hair genes to trigger its growth.

Concluding Remarks

In summary, recent findings highlighted here represent a significant step toward understanding RSL4-mediated regulation of cell size. Nevertheless, it remains to be determined how *RSL4* expression is controlled or balanced under conflicting growth signals or when plants are exposed to additive or synergistic cues. Thus, detailed studies of the effect of each individual factor and combined signals on *RSL4* expression are needed to unravel how the cell sizing process is fine-tuned. The identification and dissection of all components involved in this regulatory network (Figure 1) remain tasks for future research, and will require a concerted effort by the plant research community.

Author Contributions

J.M.E conceived the project and, with E.M., C.B., S.P.D.J., and S.M., wrote the article. E.M., C.B., and S.P.D.J. also provided technical assistance.

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protein sequence, it is under 26S proteasomal degradation, which regulates its lifetime. RSL4 integrates internal and external cues by triggering the expression of a core of RHE genes (~124 putative direct targets) to control root hair cell size. In addition, other TFs (e.g., LRL1-LRL3) also regulate root hair gene expression in an RSL4-independent manner. Abbreviations: Aux-RE, auxin responsive element; CYCP, P-type cyclins; D, D-BOX motif (RXXLXXXN); EXP, expansins; GTs, glycosyltransferases; LRL, *Lotus japonicus* Roothairless Like; LRX, leucine-rich extensin; NAGS, amino-acid acetyltransferase; PER, type-III peroxidases; PRP, proline-rich proteins; RBOH, respiratory burst oxidase homolog proteins; RHE, root hair-specific element; RIC, Rop-interactive Crib motif-containing protein; RLKs, receptor-like kinases; RNS, rinonuclease; TET, tetraspanin.