

## Article Addendum

# A family of receptor-like kinases are regulated by BES1 and involved in plant growth in *Arabidopsis thaliana*

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Plant growth is dictated by both developmental and environmental cues, many of which are perceived by receptor-like kinases (RLKs). In *Arabidopsis*, there are more than 600 RLKs; but the functions of most of them are unknown. We recently found that several members of CrRLK1L family RLKs are regulated by plant steroid hormone Brassinosteroids (BRs). Two of the RLKs, FERONIA (FER) and THESEUS1 (THE1) have been previously found to inhibit cell elongation during pollen tube/synergid cell recognition and in sensing cell wall integrity after damage, respectively. However, we found that HERCULES1 (HERK1), another member in the family, as well as THE1 and FER, are regulated by BRs and required for cell elongation during vegetative growth. Here we provide additional evidence for the regulation of the family members by BR effector protein BES1. We also show that another member in the family, designated as HERCULES2 (HERK2), functions redundantly with HERK1 and THE1 to promote stem elongation. Our results, together with those from others, provide compelling evidence that the CrRLK1L family members play important role in plant growth.

HERK1 (At3g46290), THE1 (At5g54380) and FER (At3g51550) belong to the CrRLK1L (named after the founding member identified in *Catharanthus roseus*) family RLKs that has 17 members in *Arabidopsis*.<sup>1,2</sup> Several elegant work demonstrated that FER and THE1 function to repress cellular growth in several processes.<sup>3</sup> FER is involved in male-female interaction during fertilization.<sup>4,5</sup> In wild-type plants, FER is localized in the plasma membrane of synergid cells and was proposed to perceives an unknown signal from the incoming pollen tube and to inhibit its

further growth. In loss-of-function mutants of FER gene, pollen tubes overgrow and thus cannot fertilize the egg cells, resulting in infertility and embryonic lethality.<sup>5-7</sup> THE1 was identified in a suppressor screen for cellulose-deficient mutant *cesA6*. Loss-of-function mutations in THE1 suppressed the dwarf phenotype of *cesA6* and several other cell wall mutants; THE1 was therefore proposed to inhibit cell elongation when cell wall is damaged.<sup>8</sup> In contrast to the reported inhibitory effects on cellular growth in different contexts, we recently found that HERK1, THE1 and FER are actually required for optimal cell elongation during vegetative growth.<sup>9</sup>

BRs function to regulate cell elongation and many other processes.<sup>10-13</sup> BRs signal through a membrane-localized receptor kinase BRI1 and other signaling components<sup>10,14-16</sup> to control BES1 and BZR1 family transcription factors, which mediate the expression of many genes for BR responses.<sup>17-20</sup> In an effort to identify and characterize BR target genes, we found that three CrRLK1L family members, HERK1, THE1 and FER, are induced by BRs.<sup>9</sup> In addition, the *herk1 the1* double and *fer* single mutants displayed a similar stunted growth phenotype due to defect in cell elongation. Public microarray data revealed that several other members in the family are also induced by Brassinolide (BL), the most active BR (<http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi>). To further confirm that these RLK genes are modulated by BRs and/or BES1, we performed a microarray experiment with *bes1-D* mutant,<sup>19</sup> a constitutive BR response mutant due to highly accumulated BES1 protein, with En-2 as wild-type control (L.L. & Y.Y. in preparation). The expression of all CrRLK1L members is examined (Fig. 1A). At least 6 of the family members, including FER, THE1, HERK1, At1g30570 (HERK2, see next), At2g39360 and At5g24010, are slightly upregulated by BL treatment in the wild-type and are more clearly increased in *bes1-D* mutant, especially in the presence of BL. The results suggest that at least these 6 genes in the CrRLK1L family are modulated by BR pathway through BES1.

To test whether other BR-regulated members are also involved in plant growth, we obtained a T-DNA knockout line for HERK2 from ABRC (SALK\_105055, *herk2*), constructed *herk1 herk2 the1* triple mutant and examined their growth phenotypes (Fig. 1B–D). At the young seedling stage, while either *herk2* or

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*herk1 the1* has only slightly reduced growth, *herk1 herk2 the1* triple mutant has clearly reduced hypocotyl elongation (Fig. 1B). Similarly, *herk1 herk2 the1* adult plants also have reduced petiole lengths compared to *herk1 the1* double mutant (Fig. 1C and D). Taken together, we conclude that HERK2 functions redundantly with HERK1 and THE1 to promote stem (hypocotyl and leaf petiole) elongation. Although *herk1 herk2 the1* triple mutant has similar hypocotyl lengths compared to *fer* mutant at seedling stage, its adult has less stunted growth phenotype than strong *fer* mutant.<sup>9</sup> It's possible that other family members, such as At2g39360 and At5g24010, function somewhat redundantly with HERK1, HERK2 and THE1 in promoting plant growth. Further construction of multiple mutant should address the possibility.

Similar to *herk1 the1* double mutant, *herk1 herk2 the1* appears to respond to BL normally (Fig. 1B), which support our previous conclusion that HERK family RLKs define a signaling pathway that cooperates with, but is largely independent of the BR pathway in controlling plant growth. Since these RLKs are also modulated by many other plant hormones and environmental conditions (<http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi>), it's possible that these RLKs function to integrate multiple signals to regulate plant growth and other responses.

In summary, BRs function through BES1 to regulate the expression of *HERK1*, *HERK2*, *THE1* and *FER* genes. Since knockdown of *FER* displays very strong growth phenotype,<sup>9</sup> which is similar to *herk1 herk2 the1* triple mutant (Fig. 1C), *FER* may function as a heterodimer partner for other RLKs. *HERK1/HERK2/THE1/FER* may perceive unidentified ligand(s) and signal through intermediate components to regulate genes for plant growth and other responses (Fig. 1E). With all the available mutants, it is now feasible to further investigate the functions of this family of RLKs, to identify their ligand(s) and additional components in the signal transduction pathway.

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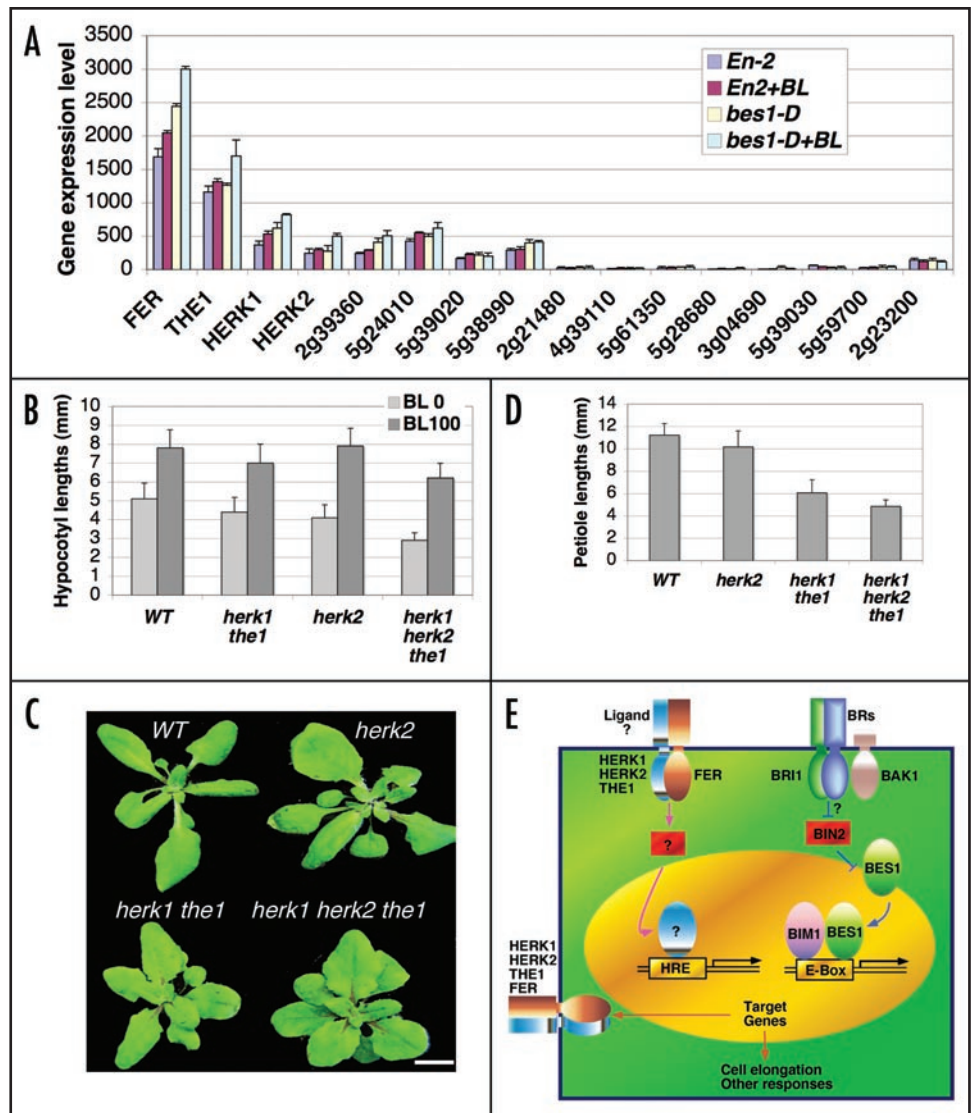


Figure 1. HERK1, HERK2, THE1 and FER are regulated by BL/BES1 and are required for cell elongation. (A) The expression of CrRLK family RLKs by BL in WT and *bes1-D* mutant. Microarrays were performed with 2-week-old seedlings with or without BL treatment (1 μM, 3 hr). At5g38990 and At5g39000 have the same probe set in Affymetrix ATH1 Genome arrays, so only the former is shown. (B) BR responses of the indicated mutants at the seedling stage, performed as described.<sup>9</sup> (C) Plant phenotypes of 24-day-old adult plants. The bar represents 10 mm. (D) Petiole lengths of the sixth leaf in WT and mutants. Averages and standard deviations from 10 plants are shown. (E) A model for HERK signaling and its crosstalk with BR pathway.

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