

Modulation of the balance between stem cell proliferation and consumption by *ERECTA*-family genes

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Stem cells in the vegetative shoot apical meristem proliferate to produce more stem cells (self-renewal) and are simultaneously consumed to form leaf primordia. Therefore, to keep a stable number of stem cells, regulation of the balance between their proliferation and consumption is important. Recently we reported that stem cell population is increased in mutant plants lacking the entire *ERECTA* (*ER*) receptor kinase family. Here we describe that loss of function of the entire *ER*-family causes a decrease in leaf number in spite of the increase in stem cell population. This suggests that stem cell consumption might be decreased in the mutant, and this could be one of reasons why stem cell population appears to be increased. This situation is in sharp contrast to *clv3* mutant, which also shows an increase in stem cell population but does not show a decrease in leaf production. We briefly discuss differences between the *er*-family mutant and the *clv3* mutant.

Loss Of Function of the Entire *ERECTA*-Family Causes a Decrease in Leaf Production in Spite of an Increase in Stem cell Population

Mechanisms that regulate the homeostasis of stem cells in the shoot apical meristem (SAM) have been intensively investigated and some important regulators have been reported.¹ *WUSCHEL* (*WUS*) is expressed at the organizing center below the stem cell region and is required for the stem cell proliferation and maintenance.^{2,3} *WUS* activates expression of *CLAVATA3*

(*CLV3*) in the stem cells,⁴ which encodes a secreted small peptide^{5,6} and serves as a useful stem cell marker gene.⁷ The secreted *CLV3* peptides in turn repress the *WUS* expression.^{5,6,8} This *WUS-CLV3* feedback loop ensures a constant number of stem cells.^{9,10}

Arabidopsis ERECTA (*ER*) receptor kinase family consists of *ER*, *ERECTA-LIKE 1* (*ERL1*) and *ERECTA-LIKE 2* (*ERL2*)^{11,12} and play significant roles in various aspects of plant development.^{11,13,14} Previously we reported that the *ER*-family members are redundantly involved in phenotypes of a mutant displaying SAM-related defects,^{15,16} and our recent paper demonstrated that they redundantly regulate stem cell homeostasis.¹⁷ *CLV3* expression domain was expanded in the *er erl1 erl2* triple mutant (hereafter, *er*-family mutant), indicating that stem cell population was increased in the mutant. Interestingly, though the *CLV3* expression was highly upregulated, *WUS* expression did not show such remarkable change. In this mutant, the SAM morphology was flattened and broadened compared with the wild-type SAM (Fig. 1A and B).¹⁷ In addition to this defect in the SAM morphology, the number of formed leaves appeared to be decreased in the *er*-family mutant (Fig. 1A and B, triangles). This decrease in leaf production might indicate that stem cell consumption to form leaves is slower in the *er*-family mutant than in wild-type plants. Given that the balance between stem cell proliferation and consumption is important to determine the stem cell population in each situation, the slower consumption of stem cells in the *er*-family mutant could be one of reasons why

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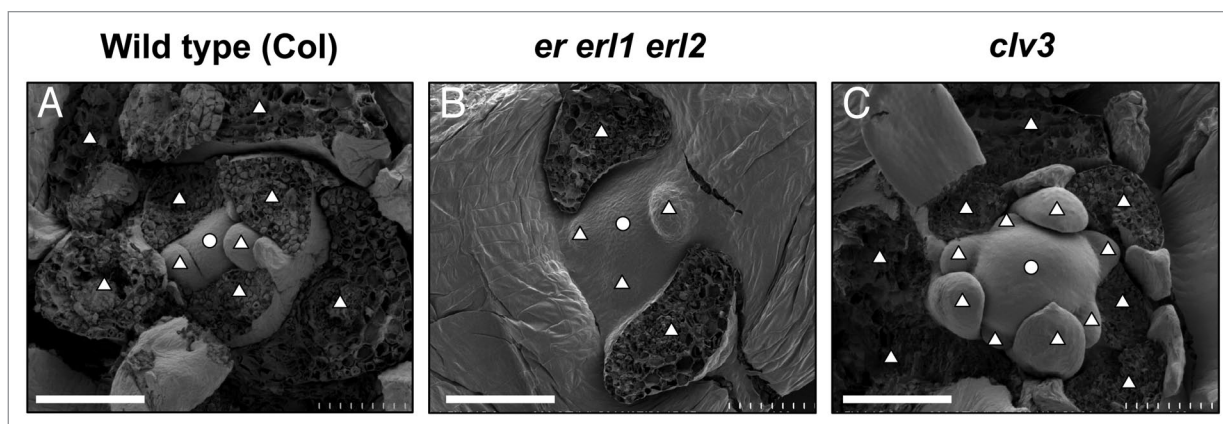


Figure 1. SEM images of shoot apex regions. The SAMs of 10-d-old seedlings with the genotypes of (A) wild type (Col), (B) *er-105 erl1-2 erl2-1* triple mutant and (C) *clv3-2* mutant were observed by SEM. Circles indicate the SAMs. Triangles indicate leaf primordia and traces of developing leaves that were removed to observe the SAM. Bars = 100 μm .

stem cell population is increased in the mutant. Detailed analysis on rates of both proliferation and consumption of stem cells in the *er*-family mutant would be an important issue in the future research.

Comparison Between the *er*-Family Mutant and the *clv3* Mutant

Loss-of-function mutations in *CLV3* induce the expansion of *WUS* expression, resulting in a dramatic increase in stem cell population.^{4,9} Because both the *er*-family mutant and the *clv3* mutant show an increase in stem cell population, we compared phenotypes of the shoot apex regions between them. The *clv3* mutant displayed a large dome-shaped SAM (Fig. 1C), which was different from a flattened SAM in the *er*-family mutant (Fig. 1B). This might be due to a difference in *WUS* expression. The *WUS* expression is drastically expanded in the *clv3* mutant,⁹ while it is not the case in the *er*-family mutant.¹⁷ Another apparent difference between the *er*-family mutant and the *clv3* mutant was related to leaf production. As described above, though the stem cell population was increased in the *er*-family mutant,¹⁷ leaf production was decreased, compared with wild-type plants (Fig. 1A and B, triangles). On the other hand, in the *clv3* mutant, leaf production was apparently increased (Fig. 1C). It is likely that, in the *clv3* mutant, the stem cell population is increased by enhanced stem cell proliferation triggered by drastic

upregulation of *WUS* expression.⁹ This is in sharp contrast to the case of the *er*-family mutant where slower consumption of stem cells due to slower leaf production could be one of reasons of increased stem cell population. However, interestingly, the stem cell proliferation was remarkably activated by cytokinin even in the *er*-family mutant and, in this cytokinin-treated condition, leaf production was also drastically stimulated,¹⁷ which was similar to the *clv3* phenotypes. It might be plausible that the *ER*-family regulates the balance between stem cell proliferation and consumption via modulation of cytokinin pathway. More detailed analysis on relationships among stem cell regulation by the *WUS-CLV3* circuit, formation of leaf primordia, and cytokinin action in the *er*-family mutant might help to understand substantial roles for the *ER*-family in the SAM regulation.

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