Stem Cell Maintenance in the Shoot Apical Meristems and during Axillary Meristem Development

Hiro-Yuki Hirano^{1*} and Wakana Tanaka^{1,2}

¹ School of Science, The University of Tokyo, 7–3–1 Hongo, Bunkyo-ku, Tokyo 113–8654, Japan

² Graduate School of Integrated Sciences for Life, Hiroshima University, 1–4–4 Kagamiyama, Higashi-Hiroshima 739–8528, Japan

Received December 26, 2019; accepted January 22, 2020

Summary In plants, the shoot apical meristem (SAM), which is formed during embryogenesis, plays a crucial role in postembryonic development of the above-ground shoots. The SAM harbors pluripotent stem cells, which provide daughter cells to differentiate lateral organs and stems. As the plant grows, the primary shoot generates the axillary meristems, which are responsible for secondary shoot development. During the development of axillary meristem, stem cells must be established and maintained to build up the axillary meristems. While the mechanism underlying stem cell maintenance in the SAM, including the WUSCHEL-CLAVATA negative feedback loop, has been well elucidated, studies on the regulation of stem cell maintenance during axillary meristem development have only just begun. In this minireview, we describe the genetic mechanisms underlying stem cell maintenance in both the established SAM and the developing axillary meristem in two model plants, *Arabidopsis thaliana* and *Oryza sativa* (rice). We also discuss the function of genes that are specific or shared between the established and developing meristems or between *Arabidopsis* and rice.

Keywords Axillary meristem, *CLAVATA*, Rice, Shoot apical meristem, Stem cell, *WUSCHEL*.

Plant architecture is determined by various morphological traits, such as the number and pattern of branches, size and shape of leaves, morphology of flowers, and type of inflorescence (Yamaguchi *et al.* 2010, Nakayama *et al.* 2012, Dhaliwal *et al.* 2018, Kumar *et al.* 2018, Nasirvand *et al.* 2018, Shiogai *et al.* 2018, reviewed in Bell 2008, Soltis and Soltis 2014, Tsukaya 2018). The branches are formed from the axillary buds, which develop in the axil of the leaves. Branch formation comprises two developmental stages: (i) initiation and establishment of the axillary bud in the leaf axil, and (ii) outgrowth of the axillary shoot from the bud. A large number of genes responsible for transcription and signaling, in addition to phytohormones such as auxin and strigolactone, regulate these two developmental events (reviewed in Domagalska and Leyser 2011, Wang *et al.* 2016, 2018, Barbier *et al.* 2019). Accumulating studies on hormone interactions and genetic networks have provided deep understanding of the mechanism that regulates axillary bud growth. By contrast, the molecular genetic mechanisms underlying the initiation and establishment of the axillary bud remain to be elucidated in detail.

The axillary bud comprises the axillary meristem and leaf primordia. The axillary meristem contains pluripo-

tent stem cells, which supply cells for the differentiation of leaf primordia, similar to the SAM. In this review, we focus on recent studies on the initiation and development of the axillary meristem and on stem cell maintenance during axillary bud formation in two model plants, *A. thaliana* (*Arabidopsis*) and *O. sativa* (rice).

Stem cell maintenance in the SAM in *Arabidopsis*

The SAM is established during embryogenesis and plays a critical role in post-embryonic development in plants. The SAM has several functional domains, such as the central, peripheral, and rib regions. In the apex of the central region, stem cells are preserved and reproduce by cell division. These stem cells also supply daughter cells to the peripheral region, where the cell fate is determined to differentiate leaf primordia. The balance between stem cell proliferation and cell supply for leaf differentiation is essential to maintain a constant population of stem cells.

This balance, termed stem cell homeostasis, is controlled by the WUSCHEL-CLAVATA (WUS-CLV) negative feedback loop in *Arabidopsis* (Fig. 1A) (reviewed in Ha *et al.* 2010, Somssich *et al.* 2016, Fletcher 2018). The *WUS* gene, encoding a WOX-type transcription factor, promotes stem cell proliferation and the expression of *CLV3*. *WUS* is expressed in the lower part of the central region, called the organizing center, whereas *CLV3*

* Corresponding author, e-mail: hyhirano@bs.s.u-tokyo.ac.jp
DOI: 10.1508/cytologia.85.3

Fig. 1. Schematic representation of the regulation of stem cell maintenance in Arabidopsis (A) and rice (B, C).

is expressed in the stem cell region. Therefore, *WUS* acts non-cell autonomously to promote stem cell proliferation and *CLV3* induction. This non-cell autonomous action of *WUS* is explained by the movement of *WUS* protein from the organizing center to the stem cell region (Yadav *et al.* 2011). An apical–basal gradient of HAIRY MERISTEM (HAM) proteins confines expression of *CLV3* to the apical region of the SAM (Zhou *et al.* 2018). HAMs prevent activation of *CLV3* by interacting with *WUS* in the organizing center where the HAM level is high, whereas *WUS* induces *CLV3* expression in the apical region where the HAM level is low.

CLV3 encodes a small secreted protein with a CLE domain, which is processed into a small peptide of 13 amino acids, called a *CLV3*-CLE peptide (Kondo *et al.* 2006). The *CLV3*-CLE peptide further undergoes chemical modification, including proline hydroxylation and hydroxyproline arabinosylation (Ohya *et al.* 2009). The modified *CLV3*-CLE peptide is perceived by *CLV1*, an LRR-type receptor kinase, whose expression domain encompasses the organizing center, that is, *WUS* expression domain (Ogawa *et al.* 2008). The *CLV3* signal ultimately represses *WUS* expression. Thus, *CLV3* negatively regulates stem cell proliferation through the repression of *WUS*. Accordingly, stem cells are maintained at an almost constant number by this *WUS*-*CLV* negative feedback loop.

Stem cell maintenance during axillary meristem development in Arabidopsis

The axillary meristem develops in the leaf axil and, after its establishment, preserves stem cells in the apical region of its central region, similar to the SAM. Leaf primordia are differentiated from the axillary meristem and form axillary buds together with the meristem. After outgrowth of the bud, the axillary meristem acts as the SAM in the secondary shoot (branch).

On the basis of morphological studies, two alternative models have been proposed for the origin of the axillary meristem (Steeves and Sussex 1989). In the ‘detached meristem’ model, a few pluripotent undifferentiated cells

are detached from the primary SAM and preserved in the leaf axil. The axillary meristem develops later from these cells. Alternatively, in the ‘*de novo* induction’ model, differentiated cells are re-specified as undifferentiated meristematic cells, which are subsequently used to form the axillary meristem.

The observation that *SHOOT MERISTEMLESS (STM)*, a marker of undifferentiated cells in the SAM, is expressed in the leaf axil before initiation of the axillary meristem supports the former model, but is not sufficient to exclude the latter model (Long and Barton 2000, Greb *et al.* 2003). Recent studies using live cell imaging have clearly shown that stem cells derived from the SAM are progenitors of the axillary meristems in Arabidopsis and tomato, supporting the ‘detached meristem’ model (Burian *et al.* 2016, Shi *et al.* 2016). In addition, Burian *et al.* (2016) have shown that the progenitor cells are maintained in a quiescent state after being separated from the stem cell pool in the SAM to minimize mutation by DNA replication. Shi *et al.* (2016) confirmed the importance of the *STM*-expressing cells in the leaf axil by a laser ablation experiment, and revealed that upregulation of *STM* expression by *REVOLUTA (REV)* is required for initiation of axillary meristem development.

Cytokinin is also necessary to initiate axillary meristem development in Arabidopsis. Cytokinin signal is observed prior to an accumulation of *WUS* transcripts (Wang *et al.* 2017). Neither cytokinin signaling nor *WUS* expression is observed in the leaf axil of mutants defective in axillary bud formation, such as *lateral suppressor (las)* or *rev*. Cytokinin signaling is needed for *de novo* induction of *WUS* in the leaf axil: some type-B ARABIDOPSIS RESPONSE REGULATORS (ARRs), such as *ARR1* and *ARR2*, directly activate *WUS* expression (Wang *et al.* 2017). Consistent with this, no *WUS* expression is observed in the *arr1* mutant.

Expression of *CLV3* begins slightly but clearly later than that of *WUS* (Xin *et al.* 2017) during axillary meristem formation. Given that *WUS* promotes *CLV3* expression in the SAM, the timing of the expression of the two genes suggests that *WUS* also induces *CLV3* expression

in axillary meristem development. Interestingly, *CLV3* is expressed in the central part of the mass of undifferentiated cells that develops into the axillary meristem, and this *CLV3*-expression region overlaps substantially with the *WUS*-expression region (Xin *et al.* 2017, Zhou *et al.* 2018). Soon after, expression of *CLV3* becomes to be localized to the apical region of the mass, similar to its expression in the SAM. This localized *CLV3* expression, which is distinct from *WUS* expression, might indicate near-completion of the axillary meristem. The apical–basal gradient of HAM also confines *CLV3* expression to the apical region of the axillary meristem, similar to its function in the SAM (Zhou *et al.* 2018). In the *clv3* mutant, the expression domain of *WUS* is expanded in the axillary meristem (Xin *et al.* 2017). This is similar to the observation that *WUS* is expressed widely in the SAM of *clv3*. These observations suggest that stem cells are maintained during axillary meristem development by a genetic mechanism that is similar to the pathway regulating stem cells in the SAM.

Stem cell maintenance in established meristems in rice

The CLV-like signaling that negatively regulates stem cell proliferation in Arabidopsis is essentially conserved in established meristems such as the SAM and the flower meristem (FM) in rice (Fig. 1B, C) (reviewed in Pautler *et al.* 2013, Tanaka *et al.* 2014, Somssich *et al.* 2016, Fletcher 2018). A mutation in *FLORAL ORGAN NUMBER1* (*FON1*) or *FON2* (also called *FON4*) leads to an enlargement of both the FM and the inflorescence meristem (IM) (Suzaki *et al.* 2004, 2006, Chu *et al.* 2006, Suzuki *et al.* 2019a). *FON1* encodes a CLV1-like LRR-type receptor kinase, whereas *FON2* encodes a small protein containing the CLE domain, which is highly similar to the CLE domain of *CLV3*. The *FON2* expression domain is expanded in the enlarged FMs and IMs of the *fon1* and *fon2* mutants, suggesting that *FON2* marks stem cells in rice, similar to the function of *CLV3* in Arabidopsis (Suzaki *et al.* 2006, Suzuki *et al.* 2019a). Overexpression of *FON2* severely reduces the number of branches, spikelets (flowers), and floral organs, probably due to a decrease in stem cells in the IM and FM (Suzaki *et al.* 2006). In the *fon1* mutant, *FON2* overexpression does not bring about any obvious defects in the flower, suggesting that *FON1* act as a receptor for the *FON2*-CLE peptide. These findings indicate that *FON* signaling negatively regulates stem cell maintenance in rice, similar to CLV signaling in Arabidopsis (Fig. 1A, C).

Unlike Arabidopsis, rice has multiple pathways that negatively regulate stem cell maintenance. *FON2 SPARE1* (*FOS1*) acts redundantly with *FON2* in the FM in wild rice species and *indica* strains, but mainly lacks function in *japonica* strains due to a mutation at the processing site of the signal peptide (Suzaki *et al.* 2009). *FON2-LIKE CLE PROTEIN1* (*FCP1*) and *FCP2* have been shown to be redundantly involved in negatively regulating main-

tenance of the vegetative SAM (Fig. 1B) (Suzaki *et al.* 2008). Simultaneous downregulation of both *FCP1* and *FCP2* in seedlings causes expansion of the *FON2* expression domain in the SAM and inhibition of leaf differentiation (Ohmori *et al.* 2013). Overexpression of *FCP1* leads to termination of the SAM in regenerated shoots, unlike *FON2*, whose overexpression has relatively weak effects on shoot regeneration (Suzaki *et al.* 2008). Because the effect of *FCP1* overexpression is also observed in the *fon1* mutant, the *FCP1* signal is probably transmitted through a receptor other than *FON1*. In maize (*Zea mays*), the *FCP1* receptor has been identified as FASCIATED EAR3 (*FEA3*) (Je *et al.* 2016). A mutation in either *ZmFCP1* or *FEA3* results in a marked enlargement of the IM, and both genes function in the same genetic pathway. This *FCP1*–*FEA3* pathway has been shown to be also conserved in Arabidopsis (Je *et al.* 2016).

Unlike the CLV-like genes, the function of the *WUS* ortholog *TILLARS ABSENT1* (*TAB1*) is not conserved in rice (Tanaka *et al.* 2015, Suzuki *et al.* 2019b). In wild type, *TAB1* is not expressed in the vegetative SAM. A loss-of-function mutant of *TAB1* has been isolated by the TILLING (targeting induced local lesions in genomes) method and shows normal growth of the primary shoot (Tanaka *et al.* 2015). The *tab1* mutant has a normal SAM and produces the same number of leaves in the primary shoot as wild type. In addition, the stem cell marker *FON2* and undifferentiated cell marker *OSHI1*, an ortholog of *STM*, are expressed similarly in the SAM in both *tab1* and wild-type (Tanaka *et al.* 2015, Tanaka and Hirano 2020). Collectively, these results indicate that *TAB1* does not function in stem cell maintenance in the SAM.

Instead, another *WOX* gene, *OsWOX4*, is involved in maintenance of the SAM in rice (Fig. 1B) (Ohmori *et al.* 2013). *OsWOX4* is expressed in the various types of aerial meristem, such as the vegetative SAM, IMs, FMs, and branch meristems (BMs). RNA silencing of *OsWOX4* leads to downregulation of *FON2* and *OSHI1* in the vegetative SAM of seedlings, resulting in termination of the SAM. Similar to Arabidopsis *WUS*, *OsWOX4* promotes cytokinin signaling (Gordon *et al.* 2009, Chickarmane *et al.* 2012). In addition, *OsWOX4* is negatively regulated by *FCP1*. Therefore, the *OsWOX4*–*FCP1* pathway seems to play a role in stem cell maintenance in the SAM in rice, similar to the *WUS*–*CLV* pathway in Arabidopsis (Fig. 1A, B). *WOX4* is required for the maintenance of vascular stem cells in Arabidopsis (Hirakawa *et al.* 2010), suggesting that two orthologous genes have a similar function in stem cell maintenance but have diversified during the evolution rice and Arabidopsis to perform their functions in different meristematic tissues. In rice, *OsWOX4* is also important in early leaf development (Yasui *et al.* 2018).

Fig. 2. Genetic regulation of stem cell maintenance during axillary meristem development in rice. Upper panel, the four stages of axillary meristem development. Lower panel, the two pathways involved in regulation of stem cell maintenance. eAM, established axillary meristem; lp, leaf primordia; pp, prophyll.

Stem cell maintenance during axillary meristem development in rice

Although not required for stem cell maintenance in the SAM, *TAB1* is essential for the formation of axillary shoots (called tillers) in rice (Tanaka *et al.* 2015). The requirement of the *WUS* ortholog (also called *MONOCULMI* and *STERILE AND REDUCED TILLERING1*) for tiller formation is supported by the studies on gene cloning using reduced tiller mutants (Lu *et al.* 2015, Mjomba *et al.* 2016).

Formation of the axillary bud is compromised in the *tab1/moc3* mutant (Lu *et al.* 2015, Tanaka *et al.* 2015). In wild type, the axillary bud is formed on the culm (stem) near the boundary region between the culm and the leaf primordium. In the *tab1* mutant, the progress of axillary bud formation is arrested at various developmental stages due to a failure of axillary meristem development (Tanaka *et al.* 2015). In wild-type rice, a dome-like axillary meristem (similar to the shape of the SAM) is formed *via* a premeristem zone, where a transient meristem differentiates the primordium of the prophyll, a special leaf enclosing the axillary bud. *TAB1* is expressed in this premeristem zone (Fig. 2A). Whereas *OSH1* is expressed in the premeristem zone and in subsequent developing meristems in wild type (stage 1 and stage 2), its expression is strongly reduced or absent in *tab1*. Therefore, *TAB1* seems to maintain cells in an undifferentiated state in the premeristem zone. Interestingly, *TAB1* expression is transient and disappears at the later stages of axillary meristem development (stage 3 and stage 4). At the same time, *WOX4* starts to be expressed instead of *TAB1* (Fig. 2A). Thus, two related *WOX* genes, *TAB1* and *WOX4*, sequentially promote development of the

axillary meristem in rice (Fig. 2B). *OSH1* is also plays an essential role in axillary meristem development, because axillary bud formation is profoundly affected in the *osh1* mutant (Tanaka *et al.* 2019).

Expression of the stem cell marker *FON2* is observed at the initial stages of axillary meristem development at almost the same time as the appearance of the bulge of the premeristem zone (Fig. 2A, stage 1) (Tanaka and Hirano 2020). This suggests that stem cell identity is established very early in development of the axillary meristem. *FON2* is expressed at the apical region of the premeristem zone from stage 1; this early localization of *FON2* at the apical region contrasts with the localization of Arabidopsis *CLV3*, which alters its expression pattern during the course of axillary meristem development (Xin *et al.* 2017, Zhou *et al.* 2018). Expression of *FON2* is not observed in *tab1*, suggesting that *TAB1* induces *FON2* and that stem cells are not maintained in the absence of *TAB1* (Tanaka and Hirano 2020). *MOC1* and *LAX PANICE1* (*LAX1*), which are required for axillary bud formation, are expressed normally in *tab1*, suggesting that *TAB1* functions downstream of these two genes (Komatsu *et al.* 2003, Li *et al.* 2003, Tanaka and Hirano 2020). In addition, axillary meristem development is initiated in *tab1*, although developmental arrest occurs soon afterwards. Overall, the absence of *FON2* expression in *tab1* strongly suggests that *TAB1* is required to maintain, but not initiate, stem cells during axillary meristem development.

In the *fon2* mutant, the size of the premeristem zone is slightly enlarged and the expression domain of *FON2* is expanded as compared with wild type. Therefore, it seems likely that stem cell proliferation is negatively

regulated by *FON2*. Consistent with this idea, overexpression of *FON2* partially inhibits meristem maintenance during axillary meristem development, resulting in a reduction in tiller number (Tanaka and Hirano 2020). *TAB1* expression is elevated in *fon2*. Accordingly, the genetic relationship between *TAB1* and *FON2* in axillary meristem development in rice is highly similar to that of *WUS* and *CLV3* in the SAM in Arabidopsis (Figs. 1A, 2B).

The *tab1 fon2* double mutant generates tillers to some extent, suggesting that the *fon2* mutation partially suppresses the *tab1* phenotype (Tanaka and Hirano 2020). This genetic interaction between *TAB1* and *FON2* is in high contrast to that between *WUS* and *CLV3* in Arabidopsis, where the *wus clv3* double mutant exhibits the same phenotype as the *wus* single mutant, indicating that *wus* is completely epistatic to *clv3* (Laux *et al.* 1996). The enigmatic phenotype of the *tab1 fon2* mutant can be explained by the observation that *WOX4* is expressed precociously in the premeristem zone in the absence of *FON2* (Tanaka and Hirano 2020). Because the induction of *WOX4* by the *TAB1* promoter in *tab1* rescues its mutant phenotype, precociously expressed *WOX4* seems to function in place of *TAB1* during axillary bud formation in the *tab1 fon2* double mutant.

In summary, *WOX4* promotes stem cells in the SAM, whereas *TAB1* promotes them during axillary meristem development. Similarly, *FCP1* negatively regulates stem cell proliferation in the SAM, whereas *FON2* negatively regulates it during axillary bud formation. Thus, a similar genetic mechanism regulates stem cell maintenance in both the established meristem and the developing meristem during axillary bud formation. A similar mechanism involving *WUS* and *CLV3* functions in SAM maintenance and in axillary meristem development in Arabidopsis, as described above. In rice, however, the involvement of *WOX4* seems to make the mechanism underlying stem cell maintenance more complex as compared with Arabidopsis.

Future perspective

Recent studies demonstrate that the fundamental mechanism underlying stem cell maintenance is conserved in Arabidopsis and rice, two relatively distant species among angiosperms. However, the specific genes and their contribution differ between the two species: for example, the function of *WUS* to regulate stem cell maintenance in both the SAM and developing meristems in Arabidopsis has been divided between two genes, *TAB1* and *WOX4*, in rice. In legumes such as *Lotus japonicus*, the gene that is orthologous to Arabidopsis *CLV1* and rice *FON1* does not have a role in stem cell maintenance, but regulates the number of nodules (Nishimura *et al.* 2002). Therefore, further studies using more varied species are needed to understand the conserved mechanisms underlying stem cell maintenance among angiosperms and the

mechanisms specific to each species.

Acknowledgements

This study was supported by MEXT/JSPS KAKENHI (17H03745 to H.-Y. H.; 19K16160 to W. T.).

References

- Barbier, F. F., Dun, E. A., Kerr, S. C., Chabikwa, T. G. and Beveridge, C. A. 2019. An update on the signals controlling shoot branching. *Trends Plant Sci.* **24**: 220–236.
- Bell, A. D. 2008. *Plant Form: An Illustrated Guide to Flowering Plant Morphology*. Timber Press, Portland.
- Burian, A., Barbier de Reuille, P. and Kuhlemeier, C. 2016. Patterns of stem cell divisions contribute to plant longevity. *Curr. Biol.* **26**: 1385–1394.
- Chickarmane, V. S., Gordon, S. P., Tarr, P. T., Heisler, M. G. and Meyerowitz, E. M. 2012. Cytokinin signaling as a positional cue for patterning the apical-basal axis of the growing Arabidopsis shoot meristem. *Proc. Natl. Acad. Sci. U.S.A.* **109**: 4002–4007.
- Chu, H., Qian, Q., Liang, W., Yin, C., Tan, H., Yao, X., Yuan, Z., Yang, J., Huang, H., Luo, D., Ma, H. and Zhang, D. 2006. The *FLORAL ORGAN NUMBER4* gene encoding a putative ortholog of Arabidopsis CLAVATA3 regulates apical meristem size in rice. *Plant Physiol.* **142**: 1039–1052.
- Dhaliwal, A., Dhaliwal, R. S., Kaur, N. and Gupta, R. C. 2018. Cytomorphological study in genus *Cenchrus* L.: An important medicinal plant from north India (family: Poaceae). *Cytologia* **83**: 45–52.
- Domagalska, M. A. and Leyser, O. 2011. Signal integration in the control of shoot branching. *Nat. Rev. Mol. Cell Biol.* **12**: 211–221.
- Fletcher, J. C. 2018. The CLV-WUS stem cell signaling pathway: A roadmap to crop yield optimization. *Plants* **7**: E87.
- Gordon, S. P., Chickarmane, V. S., Ohno, C. and Meyerowitz, E. M. 2009. Multiple feedback loops through cytokinin signaling control stem cell number within the Arabidopsis shoot meristem. *Proc. Natl. Acad. Sci. U.S.A.* **106**: 16529–16534.
- Greb, T., Clarenz, O., Schafer, E., Muller, D., Herrero, R., Schmitz, G. and Theres, K. 2003. Molecular analysis of the *LATERAL SUPPRESSOR* gene in Arabidopsis reveals a conserved control mechanism for axillary meristem formation. *Genes Dev.* **17**: 1175–1187.
- Ha, C. M., Jun, J. H. and Fletcher, J. C. 2010. Shoot apical meristem form and function. *Curr. Top. Dev. Biol.* **91**: 103–140.
- Hirakawa, Y., Kondo, Y. and Fukuda, H. 2010. TDIF peptide signaling regulates vascular stem cell proliferation via the WOX4 homeobox gene in Arabidopsis. *Plant Cell* **22**: 2618–2629.
- Je, B. I., Gruel, J., Lee, Y. K., Bommert, P., Arevalo, E. D., Eveland, A. L., Wu, Q., Goldshmidt, A., Meeley, R., Bartlett, M., Komatsu, M., Sakai, H., Jonsson, H. and Jackson, D. 2016. Signaling from maize organ primordia via FASCIATED EAR3 regulates stem cell proliferation and yield traits. *Nat. Genet.* **48**: 785–791.
- Komatsu, K., Maekawa, M., Ujiie, S., Satake, Y., Furutani, I., Okamoto, H., Shimamoto, K. and Kyoizuka, J. 2003. *LAX* and *SPA*: Major regulators of shoot branching in rice. *Proc. Natl. Acad. Sci. U.S.A.* **100**: 11765–11770.
- Kondo, T., Sawa, S., Kinoshita, A., Mizuno, S., Kakimoto, T., Fukuda, H. and Sakagami, Y. 2006. A plant peptide encoded by *CLV3* identified by in situ MALDI-TOF MS analysis. *Science* **313**: 845–848.
- Kumar, A., Meena, H. S., Ram, B., Priyamedha, Sharma, A., Yadav, S., Singh, V. V. and Rai, P. K. 2018. Some cytomorphological evidence for synthesis of interspecific hybrids between *Brassica*

- juncea* and autotetraploid *B. fruticulosa*. Cytologia **83**: 421–426.
- Laux, T., Mayer, K. F. X., Berger, J. and Jürgens, G. 1996. The *WUSCHEL* gene is required for shoot and floral meristem integrity in *Arabidopsis*. Development **122**: 87–96.
- Li, X., Qian, Q., Fu, Z., Wang, Y., Xiong, G., Zeng, D., Wang, X., Liu, X., Teng, S., Hiroshi, F., Yuan, M., Luo, D., Han, B. and Li, J. 2003. Control of tillering in rice. Nature **422**: 618–621.
- Long, J. and Barton, M. K. 2000. Initiation of axillary and floral meristems in *Arabidopsis*. Dev. Biol. **218**: 341–353.
- Lu, Z., Shao, G., Xiong, J., Jiao, Y., Wang, J., Liu, G., Meng, X., Liang, Y., Xiong, G., Wang, Y. and Li, J. 2015. *MONOCULM 3*, an ortholog of *WUSCHEL* in rice, is required for tiller bud formation. J. Genet. Genomics **42**: 71–78.
- Mjomba, F. M., Zheng, Y., Liu, H., Tang, W., Hong, Z., Wang, F. and Wu, W. 2016. Homeobox is pivotal for OsWUS controlling tiller development and female fertility in rice. G3 **6**: 2013–2021.
- Nakayama, H., Yamaguchi, T. and Tsukaya, H. 2012. Acquisition and diversification of cladodes: Leaf-like organs in the genus *Asparagus*. Plant Cell **24**: 929–940.
- Nasirvand, S., Zakaria, R. A., Zare, N. and Esmailipoor, B. 2018. Polyploidy induction in parsley (*Petroselinum crispum* L.) by colchicine treatment. Cytologia **83**: 393–396.
- Nishimura, R., Hayashi, M., Wu, G. J., Kouchi, H., Imaizumi-Anraku, H., Murakami, Y., Kawasaki, S., Akao, S., Ohmori, M., Nagasawa, M., Harada, K. and Kawaguchi, M. 2002. HAR1 mediates systemic regulation of symbiotic organ development. Nature **420**: 426–429.
- Ogawa, M., Shinohara, H., Sakagami, Y. and Matsubayashi, Y. 2008. Arabidopsis CLV3 peptide directly binds CLV1 ectodomain. Science **319**: 294.
- Ohmori, Y., Tanaka, W., Kojima, M., Sakakibara, H. and Hirano, H. Y. 2013. *WUSCHEL-RELATED HOMEBOX4* is involved in meristem maintenance and is negatively regulated by the CLE gene *FCPI* in rice. Plant Cell **25**: 229–241.
- Ohyama, K., Shinohara, H., Ogawa-Ohnishi, M. and Matsubayashi, Y. 2009. A glycopeptide regulating stem cell fate in *Arabidopsis thaliana*. Nat. Chem. Biol. **5**: 578–580.
- Pautler, M., Tanaka, W., Hirano, H. Y. and Jackson, D. 2013. Grass meristems I: Shoot apical meristem maintenance, axillary meristem determinacy and the floral transition. Plant Cell Physiol. **54**: 302–312.
- Shi, B., Zhang, C., Tian, C., Wang, J., Wang, Q., Xu, T., Xu, Y., Ohno, C., Sablowski, R., Heisler, M. G., Theres, K., Wang, Y. and Jiao, Y. 2016. Two-step regulation of a meristematic cell population acting in shoot branching in *Arabidopsis*. PLoS Genet. **12**: e1006168.
- Shiogai, S., Tamotsu, S. and Sakai, A. 2018. C₃-like photosynthetic properties of senescing maize leaves are accompanied by preferential senescence of mesophyll cells. Cytologia **83**: 387–391.
- Soltis, P. S. and Soltis, D. E. 2014. Flower Diversity and Angiosperm Diversification. In: Fornara, F. (ed.). The Molecular Genetics of Floral Transition and Flower Development. Elsevier, Amsterdam. pp. 221–262.
- Somssich, M., Je, B. I., Simon, R. and Jackson, D. 2016. CLAVATA-WUSCHEL signaling in the shoot meristem. Development **143**: 3238–3248.
- Steeves, T. A. and Sussex, I. M. 1989. Patterns in Plant Development. Cambridge University Press, Cambridge, UK.
- Suzaki, T., Ohneda, M., Toriba, T., Yoshida, A. and Hirano, H.-Y. 2009. *FON2 SPARE1* redundantly regulates floral meristem maintenance with *FLORAL ORGAN NUMBER2* in rice. PLoS Genet. **5**: e1000693.
- Suzaki, T., Sato, M., Ashikari, M., Miyoshi, M., Nagato, Y. and Hirano, H. Y. 2004. The gene *FLORAL ORGAN NUMBER1* regulates floral meristem size in rice and encodes a leucine-rich repeat receptor kinase orthologous to *Arabidopsis* CLAVATA1. Development **131**: 5649–5657.
- Suzaki, T., Toriba, T., Fujimoto, M., Tsutsumi, N., Kitano, H. and Hirano, H. Y. 2006. Conservation and diversification of meristem maintenance mechanism in *Oryza sativa*: Function of the *FLORAL ORGAN NUMBER2* gene. Plant Cell Physiol. **47**: 1591–1602.
- Suzaki, T., Yoshida, A. and Hirano, H.-Y. 2008. Functional diversification of CLAVATA3-related CLE proteins in meristem maintenance in rice. Plant Cell **20**: 2049–2058.
- Suzuki, C., Tanaka, W. and Hirano, H. Y. 2019a. Transcriptional corepressor ASP1 and CLV-like signaling regulate meristem maintenance in rice. Plant Physiol. **180**: 1520–1534.
- Suzuki, C., Tanaka, W., Tsuji, H. and Hirano, H. Y. 2019b. *TILLERS ABSENT1*, the *WUSCHEL* ortholog, is not involved in stem cell maintenance in the shoot apical meristem in rice. Plant Signal. Behav. **14**: e1640565.
- Tanaka, W. and Hirano, H. Y. 2020. Antagonistic action of *TILLERS ABSENT1* and *FLORAL ORGAN NUMBER2* regulates stem cell maintenance during axillary meristem development in rice. New Phytol. **225**: 974–984.
- Tanaka, W., Ohmori, Y., Ushijima, T., Matsusaka, H., Matsushita, T., Kumamaru, T., Kawano, S. and Hirano, H.-Y. 2015. Axillary meristem formation in rice requires the *WUSCHEL* ortholog *TILLERS ABSENT1*. Plant Cell **27**: 1173–1184.
- Tanaka, W., Toriba, T. and Hirano, H.-Y. 2014. Flower Development in Rice. In: Fornara, F. (ed.). The Molecular Genetics of Floral Transition and Flower Development. Elsevier, Amsterdam. pp. 221–262.
- Tanaka, W., Tshuda, K. and Hirano, H.-Y. 2019. Class I KNOX gene *OSHI* is indispensable for axillary meristem development in rice. Cytologia **84**: 343–346.
- Tsukaya, H. 2018. Leaf shape diversity with an emphasis on leaf contour variation, developmental background, and adaptation. Semin. Cell Dev. Biol. **79**: 48–57.
- Wang, B., Smith, S. M. and Li, J. 2018. Genetic regulation of shoot architecture. Annu. Rev. Plant Biol. **69**: 437–468.
- Wang, J., Tian, C., Zhang, C., Shi, B., Cao, X., Zhang, T. Q., Zhao, Z., Wang, J. W. and Jiao, Y. 2017. Cytokinin signaling activates *WUSCHEL* expression during axillary meristem initiation. Plant Cell **29**: 1373–1387.
- Wang, Q., Hasson, A., Rossmann, S. and Theres, K. 2016. *Divide et impera*: Boundaries shape the plant body and initiate new meristems. New Phytol. **209**: 485–498.
- Xin, W., Wang, Z., Liang, Y., Wang, Y. and Hu, Y. 2017. Dynamic expression reveals a two-step patterning of *WUS* and *CLV3* during axillary shoot meristem formation in *Arabidopsis*. J. Plant Physiol. **214**: 1–6.
- Yadav, R. K., Perales, M., Gruel, J., Girke, T., Jönsson, H. and Reddy, G. V. 2011. WUSCHEL protein movement mediates stem cell homeostasis in the *Arabidopsis* shoot apex. Genes Dev. **25**: 2025–2030.
- Yamaguchi, T., Yano, S. and Tsukaya, H. 2010. Genetic framework for flattened leaf blade formation in unifacial leaves of *Juncus prismatocarpus*. Plant Cell **22**: 2141–2155.
- Yasui, Y., Ohmori, Y., Takebayashi, Y., Sakakibara, H. and Hirano, H.-Y. 2018. *WUSCHEL-RELATED HOMEBOX4* acts as a key regulator in early leaf development in rice. PLoS Genet. **14**: e1007365.
- Zhou, Y., Yan, A., Han, H., Li, T., Geng, Y., Liu, X. and Meyerowitz, E. M. 2018. HAIRY MERISTEM with WUSCHEL confines CLAVATA3 expression to the outer apical meristem layers. Science **361**: 502–506.