WUSCHEL in the shoot apical meristem: old player, new tricks

Filipa Lopes¹, Carlos Galvan-Ampudia^{2*}, Benoit Landrein^{2*}

¹ Plant Stress Signaling, Instituto Gulbenkian de Ciência, Rua da Quinta Grande 6, 2780-156 Oeiras, Portugal.

² Laboratoire Reproduction et Développement des Plantes, Université de Lyon, Ecole Normale Supérieure de Lyon, UCB Lyon 1, CNRS, INRAE, Lyon Cedex, France.

* Correspondance : benoit.landrein@ens-lyon.fr, carlos.galvan-ampudia@ens-lyon.fr

Abstract:

The maintenance of the stem cell niche in the shoot apical meristem, the structure that generates all of the aerial organs of the plant, relies on a canonical feedback loop between *WUSCHEL* (*WUS*) and *CLV3* (*CLV3*). *WUS* is a homeodomain transcription factor expressed in the organizing center that moves to the central zone to promote stem cell fate. *CLAVATA3* is a peptide whose expression is induced by *WUS* in the central zone that can move back to the organizing center to inhibit *WUS* expression. Within the last 20 years since the initial formulation of the *CLV/WUS* feedback loop, the mechanisms of stem cell maintenance have been intensively studied and the function of *WUS* has been redefined. In this review, we will highlight the most recent advances in our comprehension of the molecular mechanisms of *WUS* function, of its interaction with other transcription factors and with hormonal signals and of its connection to environmental signals. Through this, we will show how *WUS* can integrate both internal and external cues to adapt meristem function to the plant environment.

Keywords (alphabetically ordered): Auxin, *CLAVATA*, Cytokinins, Light, Nitrate, Oxygen, Shoot apical meristem, *WUSCHEL*

Highlights: This review highlights recent advances in our comprehension of the molecular mechanisms of *WUSCHEL* function in stem cell maintenance in *Arabidopsis* meristem and its interaction with hormonal and environmental signals.

Introduction:

Plants generate organs throughout all their life thanks to the maintenance of stem cell niches localized in specialized tissues referred as meristems. The shoot apical meristem (SAM) is a highly organized structure, which is responsible for the generation of all of the aerial organs of the plants (Barton, 2010). Meristematic cells are characterized by the expression of *SHOOTMERISTEM LESS* (*STM*), which encodes a homeodomain transcription factor (TF) whose activity is necessary for SAM maintenance (Long *et al.*, 1996). Stem cells are located at the centre of the SAM in the central zone (CZ) (Fig. 1). They are growing and dividing relatively slowly and are marked by the expression of the *CLAVATA3* (*CLV3*) gene (Fletcher, 1999). The organizing centre (OC) is located below the central zone at the tip of the shoot meristem (RM) and is defined by the expression of the stem cell regulator *WUSCHEL* (*WUS*) (Mayer *et al.*, 1998). Cells that are advected away from the central zone through growth and division join the peripheral zone (PZ) where growth is more pronounced and where organs are initiated following specific patterns of phyllotaxis (Barton, 2010). The SAM can also be organized in layers (L1, L2 and L3) based on cell lineage analysis and characteristic cell division orientations (Poethig, 1987) (Fig. 1).

More than 20 years ago, the maintenance of the stem cell pool in the SAM of the model plant *Arabidopsis* was proposed to be controlled by a feedback loop between *WUS* and *CLV3* (Schoof *et al.*, 2000; Brand, 2000) (Fig. 1). *WUS* is a mobile homeodomain transcription factor expressed in the OC that can move to the CZ to promote stem cell fate, notably by repressing differentiation (Yadav *et al.*, 2011; Daum *et al.*, 2014). Plants lacking *WUS* expression are unable to maintain their stem cell niche in the SAM, which leads to termination of the meristem after the production of a very limited number of organs (Laux *et al.*, 1996). *CLV3* is a small peptide whose expression is induced by *WUS* in the CZ but that can repress *WUS* expression (Brand *et al.*, 2002). Plants lacking *CLV3* expression generate very large meristems producing many organs because of a lack of inhibition of *WUS* expression (Clark *et al.*, 1995; Fletcher, 1999). Several *CLV3* receptors including *CLV1*, *CLV2*, *CRN* (*CORYNE*) co-receptors, members of the *BAM* (*BARELY ANY MERISTEM*) family as well as the recently-characterized members of the *CIK* (*CLAVATA3 INSENSITIVE RECEPTOR KINASES*) family, have been isolated (Clark *et al.*, 1993; Jeong *et al.*, 1999; DeYoung *et al.*, 2006; Müller *et al.*, 2008; Nimchuk *et al.*, 2015; Hu *et al.*, 2018). Expressed in the OC but also in different domains in the SAM, they act in concert to control *WUS* expression by forming a variety of homo and hetero-dimers. The binding of *CLV3* to *CLV1* was also shown to trigger the internalization of the receptor (Nimchuk *et al.*, 2011), a mechanism that could explain the buffering effects observed following enhancement of *CLV3* expression (Müller *et al.*, 2006). As for other receptor kinases, activation of *CLV1* induces a cascade of *MAP KINASE* (*MITOGEN ACTIVATED PROTEIN*) activation ultimately leading to *WUS* repression through mechanisms that still need to be finely dissected (Betsuyaku *et al.*, 2011). *WUS* also represses the expression of *CLV1* through direct binding to its promoter thus adding another layer complexity to the core *CLV/WUS* feedback loop (Busch *et al.*, 2010). *CLV* signalling also affect auxin-mediated growth in flora primordia, notably in response to cold (Jones *et al.*, 2020).

Many studies have also pushed further our comprehension of the mechanisms regulating the expression of *WUS* and its function in the SAM. In this review, we will discuss recent advances aiming at characterizing the molecular mechanisms of *WUS* function, its connection to hormonal signalling and its response to environmental cues in the plant model *Arabidopsis* (for a more broader view of *WUS* function in other species see (Kitagawa and Jackson, 2019; Jha *et al.*, 2020)). *WUS* is also involved in floral organ identity and in floral meristem termination, but these functions will not be discussed here (for a review on this subject, see: (Sun and Ito, 2015)).

Molecular mechanisms of *WUS* function

WUS function in the SAM relies on three distinct yet interconnected processes: its movement, its capacity to form homo and heterodimers and its binding specificity. *WUS* movement from OC to CZ is a central property of the *CLV/WUS* feedback loop but it was only confirmed 10 years after the formulation of the model (Schoof *et al.*, 2000). Using a set of translational reporter fused to various florescent proteins, Yadav and colleagues indeed showed that *WUS* could move from the OC to the CZ where it directly binds to the *CLV3* promoter and that this movement was necessary for *WUS* function (Yadav *et al.*, 2011). Following this work, Daum and colleagues showed that *WUS* movement occurred through plasmodesmata and that specific sequences encoded within the *WUS* protein could promote but also restrict *WUS* movement in the SAM (Daum *et al.*, 2014). It is probable that the movement of *WUS* through plasmodesmata is regulated by specific yet uncharacterized proteins localized at plasmodesmata, similarly to what has been observed for *STM* (Winter *et al.*, 2007).

Like other homeodomain TF, WUS was also shown to form homodimers *in vitro* and *in vivo* (Busch *et al.*, 2010; Daum *et al.*, 2014). The mechanisms of WUS homodimerization have been recently studied in more depth (Box 1). Rodriguez and colleagues identified two distinct regions that are necessary for WUS dimerization. They also propose that the dimerization could be promoted by the binding to DNA, although it may not be strictly necessary (Busch *et al.*, 2010), and that it affects the stability of the TF (Rodriguez *et al.*, 2016). A further study from the same team showed that the homodimerization occurs in a concentration-dependent manner and that it affects binding to target genes (Perales *et al.*, 2016). As WUS movement through plasmodesmata is size-dependent (Yadav *et al.*, 2011) and that one of the sequence required for homodimerization is also necessary for mobility (Rodriguez *et al.*, 2016), the formation of dimers may reduce WUS mobility (Fuchs and Lohmann, 2020)(Box 1).

WUS has been shown to bind to DNA through three different motifs: a canonical TAAT motif for homeodomain TF, a G-box like domain and a TGAA domain (Lohmann *et al.*, 2001; Yadav *et al.*, 2011; Perales *et al.*, 2016). Upon binding, WUS can act as an activator but also as a repressor thanks to the recruitment of co-repressors from the TOPLESS family (Leibfried *et al.*, 2005; Busch *et al.*, 2010). Interestingly, Perales and colleagues showed that the formation of homodimers could affect both the binding to DNA and the activity of WUS (Box 1). They proposed a model where WUS monomers would activate *CLV3* in the CZ but where WUS dimers would repress *CLV3* in the OC (Perales *et al.*, 2016). Sloan and colleagues recently obtained the structure of fragments of WUS proteins bound to different DNA sequences which allowed them to study its binding specificity further (Sloan *et al.*, 2020). They showed that WUS preferentially binds to TGAA sequences and that the dimerization allows cooperative and stabilized binding to specific repeated motifs.

In addition to the formation of homodimers, WUS can also form heterodimers with members of the HAIRY MERISTEM (HAM) family of transcription factors (Zhou *et al.*, 2015, 2018) (Box 1). The triple *ham1.2.3* mutant has a very intriguing phenotype where *CLV3* expression increases and moves from the CZ to the OC. Given that HAM is only expressed in the L3, Zhou and colleagues proposed that the formation of heterodimers between WUS and HAM prevents the induction of *CLV3* in the OC while the absence of HAM in the L1 and L2 allows the induction of *CLV3* by WUS in the CZ (Zhou *et al.*, 2018). This model is supported by two sets of computational simulations that could recapitulate both WT and mutant phenotypes (Zhou *et al.*, 2018; Gruel *et al.*, 2018). Very recently, Su and colleagues also showed that WUS physically interacts with STM. They also demonstrated that STM could bind to the *CLV3* promoter and that this binding strengthened the one of WUS through the formation of a WUS/STM heterodimers (Su *et al.*, 2020) (Box 1).

Taking together these studies, we could build a model where WUS homodimers or/and WUS/HAM heterodimers may inhibit *CLV3* expression in the OC, while WUS/STM heterodimers would induce *CLV3* expression in the CZ (Box 1). Studying the movement, DNA binding and transcriptional activity of the WUS homodimers and heterodimers should allow us to further understand how the dimerization affects WUS function in both CZ and OC.

WUS interaction with hormone signalling

Following the identification of its targets, it was shown that WUS maintains stem cell identity by repressing differentiation, and that many of the WUS targets are involved in hormone signalling (Leibfried *et al.*, 2005; Busch *et al.*, 2010; Yadav *et al.*, 2013; Ma *et al.*, 2019) (Box 2). WUS and

cytokinins (CKs) have an intricate feedback loop, where *WUS* activates CK signalling by repressing negative regulators of CK signalling of the *type-A ARR* (*ARABIDOPSIS RESPONSE REGULATOR*) family (Leibfried *et al.*, 2005). In return, CKs promote stem cell fate by inducing *WUS* expression but also by repressing *CLV1* expression (Gordon *et al.*, 2009; Buechel *et al.*, 2010; Nimchuk *et al.*, 2015). Interestingly, it was also proposed that the positioning of the *WUS* domain relies on cytokinins, and more specifically on the expression pattern of the cytokinin AHKs receptors (*ARABIDOPSIS HISTIDINE KINASES*) (Gordon *et al.*, 2009; Chickarmane *et al.*, 2012). Gruel and colleagues further supported this idea through computational studies. They showed that the diffusion of two mobile signals from the epidermis: one corresponding to active CKs, the other corresponding to an unknown molecule restricting AHKs expression to the L3, could position the *WUS* domain in the OC. Interesting scaling properties of the system were highlighted using this model as it was shown that *WUS* expression domain can scale to the size and the curvature of the SAM (Gruel *et al.*, 2016) (Box 2). Identifying the molecule controlling AHKs expression should allow testing the prediction of this model and confirming that CK signalling indeed controls the positioning of the *WUS* domain in the OC.

In addition to cytokinins, the *WUS/CLV* feedback loop has also been tightly connected to auxin signalling. Auxin accumulates at specific position in the PZ to induce organ emergence thanks to polar transport mediated by PIN1 proteins (PIN-FORMED 1) (Reinhardt *et al.*, 2000). Although auxin also accumulates in the CZ, signalling is low in this zone which is mostly insensitive to the hormone (Vernoux *et al.*, 2011). A recent paper from Ma and colleagues proposed that the maintenance of the stem cells in such a low auxin signalling state is controlled by *WUS* (Ma *et al.*, 2019) (Box 2). *WUS* notably reduces the expression of the *MONOPTEROS/AUXIN RESPONSE FACTOR 5* (*MP/ARF5*) at the CZ to lower auxin signalling. They demonstrated that *WUS* also directly regulates the expression of other genes involved in auxin signalling and response through histone de-acetylation, thus preventing cells at the CZ to differentiate. Interestingly, Galvan-Ampudia and colleagues also recently showed that newly formed auxin maxima corresponding to the future primordia are formed as protrusions originated from the CZ, but these only emerge in the boundaries of the CZ where the temporal integration of auxin concentration allows the activation of auxin signalling (Galvan-Ampudia *et al.*, 2020) (Box 2). From these studies, we could build a model where *WUS* maintains stem cell fate by limiting auxin signalling in the CZ through chromatin modification, restricting organ emergence to the PZ where auxin signalling can occur. Interestingly, we can hypothesize that this inhibition of auxin signalling by *WUS* may control the rate of organ emergence in the SAM as a result of the way organs emerge following specific patterns of phyllotaxis in the SAM (box 2).

Auxin can also feedback on stem cell homeostasis thus adding another loop to the system. It has indeed been shown that *MP/ARF5* inhibits the expression of two negative ARRs in the CZ, which could thus induce cytokinin signalling and *WUS* expression in the SAM (Zhao *et al.*, 2010). *MP/ARF5* can also repress the expression of *DORNROSCHEN/ENHANCER OF SHOOT REGENERATION 1* (*DRN/ESR1*), a positive regulator of *CLV3* expression (Luo *et al.*, 2018)(box 2). Although transcriptional repression is not through direct binding to the *CLV3* promoter, *DRN/ESR1* is required to maintain *CLV3* expression in the stem cells. However, *dnr/dnr1* double mutant does not recapitulate *clv3* mutant, suggesting that *DRN* might help to fine-tune the extension of the stem cell niche rather than determining it.

WUS and the transduction of environmental cues

In addition to internal signals, it has been recently shown that several environmental cues such as light and sugar levels, mineral nutrient availability or oxygen levels can affect meristem function through hormone signalling and the CLV/WUS loop (box 3). Light is known to modulate SAM activity through auxin and cytokinins and thus influence plant growth and development at several stages of development (Yoshida *et al.*, 2011; Pfeiffer *et al.*, 2016). Accordingly, Pfeiffer and colleagues demonstrated that light and metabolic signals converge towards the TOR pathway (TARGET OF RAPAMYCIN) to modulate WUS expression in the SAM of germinating seedlings. They further showed that this effect is, at least partly, dependent on the activity of two CK degrading enzymes from the CYTOKININ OXIDASE (CKX) family (Pfeiffer *et al.*, 2016) (box 3).

Nitrogen (N) is a major mineral nutrient for plants whose availability in the soil affects metabolism, growth, and developmental processes (Vidal *et al.*, 2020). The perception of N in different organs and the integration of this information through long-distance signalling are key for coping with changes in N availability. Although nitrate can act as a signal itself, one of the major long-distance N signalling pathways is mediated by CK (Zhang *et al.*, 2020) (box 3). SAM activity also responds to changes in nitrate availability in a cytokinin-dependent manner. Osugi and colleagues indeed showed that an increase in nitrate levels in the soil leads to the production of CK precursors by specific ISOPENTENYL TRANSFERASE (IPT) enzymes in the root and to their translocation *via* the xylem to the shoot (Osugi *et al.*, 2017). Landrein and colleagues further showed that the generation of active cytokinin by LONELY GUY (LOG) enzymes from these precursors in the SAM induces CK signalling, and WUS expression. This activation leads to stem cell proliferation and increased meristem size and organ production rate (Landrein *et al.*, 2018) (box 3).

However, the effect of nitrate on meristem function may be more complex and only partly mediated by CK signalling. Nitrate is also a major source of nitric oxide (NO), a central redox signalling molecule (Fancy *et al.*, 2017). Interestingly, keeping the balance between the different forms of reactive oxygen species (ROS), such as NO, superoxide anion ($O_2^{\cdot-}$) or hydrogen peroxide (H_2O_2), affects growth and development both in shoots and roots and is important for SAM robustness against environmental fluctuations (Foyer *et al.*, 2018). Hence, accumulating ROS species in the SAM by mutating the AtFTSH4, an ATP-dependent mitochondrial protease that counteracts accumulation of internal oxidative stress, causes meristem termination at higher temperatures (Dolzblasz *et al.*, 2016). Interestingly, the key enzymes regulating ROS metabolism have a distinct spatial distribution within the SAM and the different forms of ROS play distinct roles in each domain (Yadav *et al.*, 2009; Zeng *et al.*, 2017; Foyer *et al.*, 2018) (box 3). For example, depleting $O_2^{\cdot-}$ in the CZ lead to a decrease of WUS transcript and protein levels, CLV3 expression leading to meristem termination (Zeng *et al.*, 2017). H_2O_2 , on the other hand, is mainly present in the PZ, where it inhibits WUS expression and promotes stem cell differentiation (Zeng *et al.*, 2017). H_2O_2 production can be promoted via nitrate- or cytokinin-induced NO, through the induction of superoxide dismutase, which converts superoxide ($O_2^{\cdot-}$) into H_2O_2 , pointing for a possible role of NO in the regulation of stem cell activity (Wany *et al.*, 2018). Supporting this idea, cytokinins are required for the activation of the *CYCD3* cell cycle gene by NO, thereby promoting cell proliferation (Shen *et al.*, 2013).

Oxygen levels may also affect stem cell homeostasis in both plant and animals (Le Gac and Laux, 2019). Weits and colleagues showed that the SAM is a closed hypoxic niche using a microscale oxygen electrode (Weits *et al.*, 2019) (box 3). Consistent with this, a large number of core hypoxia-

induced genes are upregulated in the SAM when compared to juvenile leaves and their expression is downregulated when meristems are exposed to higher oxygen concentrations (80%) (Weits et al., 2019). Most importantly, increasing the levels of oxygen resulted in decreased leaf production rates, supporting the importance of hypoxia for SAM activity (Weits et al., 2019). Part of this response is mediated by LITTLE ZIPPER2 (ZPR2), which is degraded by the oxygen-dependent N-degron pathway and is thus stabilized at low O₂ (Wenkel et al., 2007; Weits et al., 2019). Several targets of ZPR2, including HD-ZIPIII transcription factors and HECATE (HEC), have been shown to be involved in stem cell regulation, notably by modulating *WUS* expression.

Recently, Wu and colleagues have shown that *WUS* was also involved in plant immunity, by acting as a molecular barrier against virus spreading at the shoot apical meristem. Upon viral infection, *WUS* protein is stabilized and actively represses the expression of methyltransferases necessary for ribosome stability, thus reducing global protein synthesis and keeping the virus out the meristem (Wu *et al.*, 2020). How *WUS* protein is stabilized upon viral infection is still an open question, however this work very interestingly show an new role of *WUS* in the protecting stem cells from infections.

Conclusion

Accumulating evidence gathered in the recent years highlighted *WUSCHEL* has a central regulator of stem cell fate and differentiation in the SAM and as a point of convergence for both internal and external signals. The recent characterization of the activity of *WUS* homodimers and heterodimers with *HAM* and *STM* has notably been a huge step forward in our understanding of *WUS* function. Thanks to that, we can hypothesize that *WUS* activity in distinct tissues (such as OC and CZ), organs and stages of development might rely on the formation of specific heterodimers with other TFs, including *HAM* and *STM*. The study of the interplay between the *WUS/CLV* loop and auxin and cytokinins also highlighted very strong connections between stem cell maintenance and hormone signalling. This work notably allowed redefining the function of *WUS* in controlling organogenesis through the inhibition of differentiation. Finally, the recent characterization of the impact of environmental signal on *WUS* expression showed that the *CLV/WUS* feedback loop is finely tuned to adapt meristem function to a variety of signals, and that hormones play a central role in this process. A key question that remains to be answered is how all of these signals can be integrated by both hormone signalling and *WUS* for plants to adapt meristem maintenance and organogenesis to their constantly changing local environment.

Acknowledgments

The work of the Landrein lab is supported by the research fund of the ENS de Lyon and by the BAP department of INRAe. The work of Filipa Lara Lopes is supported by the Fundação para a Ciência e a Tecnologia of Portugal (UIDB/04551/2020, PD/BD/128397/2017, LISBOA-01-0145-FEDER-028128, PTDC/BIA-BID/32347/2017). The work of Galvan-Ampudia is supported by the Agence Nationale de la Recherche (ANR-18-CE13-0019 2018 to Teva Vernoux).

Fig.1 Organization of the *Arabidopsis* shoot apical meristem

RM: Rib meristem, PZ: Peripheral zone, OC: Organizing centre, CZ: central zone. L1 to L3: Layer 1 to layer 3.

Box 1. Recent developments in our comprehension of *WUS* regulation of *CLV3* expression

• ***WUS* dimerization could explain its dual functions in the OC and in the CZ**

Rodriguez *et al.* (2016) and Perales *et al.* (2016) showed that *WUS* forms stable homodimers upon binding to DNA in a concentration-dependent, which affects its binding to the *CLV3* promoter. They proposed a model where *WUS* dimers negatively regulate *CLV3* expression in the OC while *WUS* monomers positively regulate *CLV3* expression in the CZ (panel A).

• ***WUS* can form heterodimers with *HAM* in the OC**

Zhou *et al.* (2018) showed that *WUS* can physically interact with members of the *HAM* family of TF, which are specifically expressed in the L3 of the SAM. They proposed a model where *WUS*/*HAM* heterodimers repress *CLV3* expression in the OC while *WUS* alone in the CZ induces *CLV3* expression (panel B).

• ***WUS* can form heterodimers with *STM* in the CZ**

Su *et al.* (2020) showed that *WUS* can physically interact with *STM* and that *STM* binding to *CLV3* promoter can enhance the stability of *WUS* binding to this promoter through the formation of heterodimer in the CZ (panel B).

Box 2. Recent developments in our comprehension of the mechanisms of *WUS* regulation of meristem function

- ***WUS* modulate auxin signalling in the CZ**

Ma *et al.* (2019) showed that *WUS* acts as a rheostat to maintain stem cells in a low auxin signalling state by modulating the expression of many genes involved in auxin signalling and response through histone acetylation.

- **Auxin maxima are produced in the CZ but only emerge in the PZ**

Galvan-Ampudia *et al.* (2020) showed that newly formed auxin maxima corresponding to the future primordia are formed as protrusions originated from the CZ but only emerge in the PZ, which could result from the inhibition of auxin signalling by *WUS* in the CZ.

- **Auxin can feedback on stem cell regulation through MP/ARF5**

Luo *et al.* (2018) showed that MP/ARF5 can repress the expression of *DRN/ESR1*, a positive regulator of *CLV3* which, thus activating *WUS* expression and stem cell fate through the inhibition of *CLV* signalling.

- **A model for *WUS* control of organogenesis in the SAM**

The emergence of new organs in the SAM results from the accumulation of auxin at specific location thanks to polar auxin transport by PIN1 proteins (Reinhardt *et al.*, 2000). This mechanism of organ positioning (phyllotaxis) has long been conceptualized by the so-called inhibitory field theory (Landrein and Vernoux, 2014). This theory states that pre-existing organs inhibit the initiation of new organs at their vicinity (by depleting auxin from the surroundings). Interestingly, theoretical work testing this theory and partly validated experimentally have shown that two key parameters could control the positioning and timing of organ emergence through inhibitory fields: the size of the inhibitory fields and the radius of the central ring on which organs are initiated (Douady and Couder, 1996; Landrein *et al.*, 2015). By combining the recent work from Ma and colleagues and from Galvan-Ampudia and colleagues, we could hypothesize that *WUS* may control this second parameter by inhibiting of auxin signalling. In such scenario, increasing *WUS* activity would inhibit auxin signalling locally but increase organogenesis globally (See below). This model, that remains to be tested, would explain the tight correlation that can be measured between *WUS* expression, meristem size (which is defined as the distance between the center of the SAM and the organs) and organogenesis rate (Landrein *et al.*, 2015, 2018).

Box 3. Influence of environmental signals on stem cell homeostasis in the SAM

- **Light and metabolic signals can modulate *WUS* expression in germinating seedlings**

Pfeiffer *et al.* (2016) showed that light and metabolic signals are integrated by the TOR to regulate *WUS* expression in the SAM of germinating seedlings. They further showed that part of this response relied on the regulation of the activity of cytokinin degrading enzymes (panel A).

- **Nitrate can modulate *WUS* expression through cytokinins**

Landrein *et al.* (2018) showed that the SAM can respond to quick changes in nitrate availability in the soil thanks to long range signalling of CK precursors, that are activated in the SAM and trigger the induction of *WUS* expression (panel B).

- **ROS can also influence stem cell homeostasis in the SAM**

Zeng *et al.* (2017) showed that ROS-metabolizing enzymes displays specific patterns of expression in the SAM. They proposed that the balance between $O_2^{\cdot-}$ and H_2O_2 is involved in stem cell maintenance and differentiation in the SAM (panel C).

- **Oxygen levels can affect stem cell homeostasis in the SAM**

Weits *et al.* (2019) showed that the stem cell niche is under hypoxic conditions and that altering oxygen levels in the meristem can affect stem cell homeostasis, notably through the activity of HD-ZIP III transcription factors. This effect is mediated through the degradation of ZPR2 by the N-degron pathway (panel D).

337 **References:**

338

339 **Barton MK.** 2010. Twenty years on: the inner workings of the shoot apical meristem, a
340 developmental dynamo. *Developmental Biology* **341**, 95–113.

341 **Betsuyaku S, Takahashi F, Kinoshita A, Miwa H, Shinozaki K, Fukuda H, Sawa S.** 2011. Mitogen-
342 Activated Protein Kinase Regulated by the CLAVATA Receptors Contributes to Shoot Apical Meristem
343 Homeostasis. *Plant and Cell Physiology* **52**, 14–29.

344 **Brand U.** 2000. Dependence of Stem Cell Fate in Arabidopsis on a Feedback Loop Regulated by CLV3
345 Activity. *Science* **289**, 617–619.

346 **Brand U, Grünewald M, Hobe M, Simon R.** 2002. Regulation of CLV3 Expression by Two Homeobox
347 Genes in Arabidopsis. *Plant Physiology* **129**, 565–575.

348 **Buechel S, Leibfried A, To JPC, Zhao Z, Andersen SU, Kieber JJ, Lohmann JU.** 2010. Role of A-type
349 ARABIDOPSIS RESPONSE REGULATORS in meristem maintenance and regeneration. *European Journal*
350 *of Cell Biology* **89**, 279–284.

351 **Busch W, Miotk A, Ariel FD, et al.** 2010. Transcriptional Control of a Plant Stem Cell Niche.
352 *Developmental Cell* **18**, 841–853.

353 **Chickarmane VS, Gordon SP, Tarr PT, Heisler MG, Meyerowitz EM.** 2012. Cytokinin signaling as a
354 positional cue for patterning the apical-basal axis of the growing Arabidopsis shoot meristem.
355 *Proceedings of the National Academy of Sciences* **109**, 4002–4007.

356 **Clark SE, Running MP, Meyerowitz EM.** 1993. CLAVATA1, a regulator of meristem and flower
357 development in Arabidopsis. *Development (Cambridge, England)* **119**, 397–418.

358 **Clark SE, Running MP, Meyerowitz EM.** 1995. CLAVATA3 is a specific regulator of shoot and floral
359 meristem development affecting the same processes as CLAVATA1. *Development* **121**, 2057.

360 **Daum G, Medzihradzsky A, Suzaki T, Lohmann JU.** 2014. A mechanistic framework for noncell
361 autonomous stem cell induction in *Arabidopsis*. *Proceedings of the National Academy of Sciences*
362 **111**, 14619–14624.

363 **DeYoung BJ, Bickle KL, Schrage KJ, Muskett P, Patel K, Clark SE.** 2006. The CLAVATA1-related BAM1,
364 BAM2 and BAM3 receptor kinase-like proteins are required for meristem function in Arabidopsis:
365 *BAM receptor kinases regulate meristem function*. *The Plant Journal* **45**, 1–16.

366 **Dolzblasz A, Smakowska E, Gola EM, Sokolowska K, Kicia M, Janska H.** 2016. The mitochondrial
367 protease AtFTSH4 safeguards Arabidopsis shoot apical meristem function. *Scientific Reports* **6**, 1–14.

368 **Douady S, Couder Y.** 1996. Phyllotaxis as a Dynamical Self Organizing Process Part II: The
369 Spontaneous Formation of a Periodicity and the Coexistence of Spiral and Whorled Patterns. *Journal*
370 *of Theoretical Biology* **178**, 275–294.

371 **Fancy NN, Bahlmann AK, Loake GJ.** 2017. Nitric oxide function in plant abiotic stress. *Plant Cell and*
372 *Environment* **40**, 462–472.

373 **Fletcher JC.** 1999. Signaling of Cell Fate Decisions by CLAVATA3 in Arabidopsis Shoot Meristems.
374 *Science* **283**, 1911–1914.

375 **Foyer CH, Wilson MH, Wright MH.** 2018. Redox regulation of cell proliferation: Bioinformatics and
 376 redox proteomics approaches to identify redox-sensitive cell cycle regulators. *Free Radical Biology*
 377 *and Medicine* **122**, 137–149.

378 **Fuchs M, Lohmann JU.** 2020. Aiming for the top: non-cell autonomous control of shoot stem cells in
 379 *Arabidopsis*. *Journal of Plant Research* **133**, 297–309.

380 **Le Gac AL, Laux T.** 2019. Hypoxia Is a Developmental Regulator in Plant Meristems. *Molecular Plant*
 381 **12**, 1422–1424.

382 **Galvan-Ampudia CS, Cerutti G, Legrand J, et al.** 2020. Temporal integration of auxin information for
 383 the regulation of patterning. *eLife* **9**.

384 **Gordon SP, Chickarmane VS, Ohno C, Meyerowitz EM.** 2009. Multiple feedback loops through
 385 cytokinin signaling control stem cell number within the *Arabidopsis* shoot meristem. *Proceedings of*
 386 *the National Academy of Sciences of the United States of America* **106**, 16529–16534.

387 **Gruel J, Deichmann J, Landrein B, Hitchcock T, Jönsson H.** 2018. The interaction of transcription
 388 factors controls the spatial layout of plant aerial stem cell niches. *npj Systems Biology and*
 389 *Applications* **4**.

390 **Gruel J, Landrein B, Tarr P, Schuster C, Refahi Y, Sampathkumar A, Hamant O, Meyerowitz EM,**
 391 **Jönsson H.** 2016. An epidermis-driven mechanism positions and scales stem cell niches in plants.
 392 *Science Advances* **2**, e1500989.

393 **Hu C, Zhu Y, Cui Y, et al.** 2018. A group of receptor kinases are essential for CLAVATA signalling to
 394 maintain stem cell homeostasis. *Nature Plants* **4**, 205–211.

395 **Jeong S, Trotochaud AE, Clark SE.** 1999. The *Arabidopsis* CLAVATA2 Gene Encodes a Receptor-like
 396 Protein Required for the Stability of the CLAVATA1 Receptor-like Kinase. *The Plant Cell* **11**, 1925–
 397 1933.

398 **Jha P, Ochatt SJ, Kumar V.** 2020. WUSCHEL: a master regulator in plant growth signaling. *Plant Cell*
 399 *Reports* **39**, 431–444.

400 **Jones DS, John A, VanDerMolen KR, Nimchuk ZL.** 2020. CLAVATA Signaling Ensures Reproductive
 401 Development in Plants across Thermal Environments. *Current Biology*, S0960982220315128.

402 **Kitagawa M, Jackson D.** 2019. Control of Meristem Size. *Annual Review of Plant Biology* **70**, 269–291.

403 **Landrein B, Formosa-Jordan P, Malivert A, Schuster C, Melnyk CW, Yang W, Turnbull C, Meyerowitz**
 404 **EM, Locke JCW, Jönsson H.** 2018. Nitrate modulates stem cell dynamics in *Arabidopsis* shoot
 405 meristems through cytokinins. *Proceedings of the National Academy of Sciences* **115**, 1382–1387.

406 **Landrein B, Refahi Y, Besnard F, Hervieux N, Mirabet V, Boudaoud A, Vernoux T, Hamant O.** 2015.
 407 Meristem size contributes to the robustness of phyllotaxis in *Arabidopsis*. *Journal of Experimental*
 408 *Botany* **66**, 1317–1324.

409 **Landrein B, Vernoux T.** 2014. Auxin, Chief Architect of the Shoot Apex. In: Zažímalová E,, In: Petrášek
 410 J,, In: Benková E, eds. *Auxin and Its Role in Plant Development*. Vienna: Springer Vienna, 191–212.

411 **Laux T, Mayer KF, Berger J, Jürgens G.** 1996. The WUSCHEL gene is required for shoot and floral
 412 meristem integrity in *Arabidopsis*. *Development (Cambridge, England)* **122**, 87–96.

413 **Leibfried A, To JPC, Busch W, Stehling S, Kehle A, Demar M, Kieber JJ, Lohmann JU.** 2005. WUSCHEL
414 controls meristem function by direct regulation of cytokinin-inducible response regulators. *Nature*
415 **438**, 1172–1175.

416 **Lohmann JU, Hong RL, Hobe M, Busch MA, Parcy F, Simon R, Weigel D.** 2001. A Molecular Link
417 between Stem Cell Regulation and Floral Patterning in *Arabidopsis*. *Cell* **105**, 793–803.

418 **Long JA, Moan EI, Medford JI, Barton MK.** 1996. A member of the KNOTTED class of homeodomain
419 proteins encoded by the STM gene of *Arabidopsis*. *Nature* **379**, 66–69.

420 **Luo L, Zeng J, Wu H, Tian Z, Zhao Z.** 2018. A Molecular Framework for Auxin-Controlled Homeostasis
421 of Shoot Stem Cells in *Arabidopsis*. *Molecular Plant* **11**, 899–913.

422 **Ma Y, Miotk A, Šutiković Z, et al.** 2019. WUSCHEL acts as an auxin response rheostat to maintain
423 apical stem cells in *Arabidopsis*. *Nature Communications* **10**.

424 **Mayer KFX, Schoof H, Haecker A, Lenhard M, Jürgens G, Laux T.** 1998. Role of WUSCHEL in
425 Regulating Stem Cell Fate in the *Arabidopsis* Shoot Meristem. *Cell* **95**, 805–815.

426 **Müller R, Bleckmann A, Simon R.** 2008. The Receptor Kinase CORYNE of *Arabidopsis* Transmits the
427 Stem Cell–Limiting Signal CLAVATA3 Independently of CLAVATA1. *The Plant Cell* **20**, 934–946.

428 **Müller R, Borghi L, Kwiatkowska D, Laufs P, Simon R.** 2006. Dynamic and Compensatory Responses
429 of *Arabidopsis* Shoot and Floral Meristems to *CLV3* Signaling. *The Plant Cell* **18**, 1188–1198.

430 **Nimchuk ZL, Tarr PT, Ohno C, Qu X, Meyerowitz EM.** 2011. Plant Stem Cell Signaling Involves Ligand-
431 Dependent Trafficking of the CLAVATA1 Receptor Kinase. *Current Biology* **21**, 345–352.

432 **Nimchuk ZL, Zhou Y, Tarr PT, Peterson BA, Meyerowitz EM.** 2015. Plant stem cell maintenance by
433 transcriptional cross-regulation of related receptor kinases. *Development (Cambridge, England)* **142**,
434 1043–1049.

435 **Osugi A, Kojima M, Takebayashi Y, Ueda N, Kiba T, Sakakibara H.** 2017. Systemic transport of trans-
436 zeatin and its precursor have differing roles in *Arabidopsis* shoots. *Nature Plants* **3**.

437 **Perales M, Rodriguez K, Snipes S, Yadav RK, Diaz-Mendoza M, Reddy GV.** 2016. Threshold-
438 dependent transcriptional discrimination underlies stem cell homeostasis. *Proceedings of the*
439 *National Academy of Sciences* **113**, E6298–E6306.

440 **Pfeiffer A, Janocha D, Dong Y, et al.** 2016. Integration of light and metabolic signals for stem cell
441 activation at the shoot apical meristem. *eLife* **5**, 1–21.

442 **Poethig RS.** 1987. Clonal Analysis of Cell Lineage Patterns in Plant Development. *American Journal of*
443 *Botany* **74**, 581.

444 **Reinhardt D, Mandel T, Kuhlemeier C.** 2000. Auxin Regulates the Initiation and Radial Position of
445 Plant Lateral Organs. *The Plant Cell* **12**, 507–518.

446 **Rodriguez K, Perales M, Snipes S, Yadav RK, Diaz-Mendoza M, Reddy GV.** 2016. DNA-dependent
447 homodimerization, sub-cellular partitioning, and protein destabilization control WUSCHEL levels and
448 spatial patterning. *Proceedings of the National Academy of Sciences* **113**, E6307–E6315.

449 **Schoof H, Lenhard M, Haecker A, Mayer KFX, Jürgens G, Laux T.** 2000. The Stem Cell Population of
 450 Arabidopsis Shoot Meristems Is Maintained by a Regulatory Loop between the CLAVATA and
 451 WUSCHEL Genes. *Cell* **100**, 635–644.

452 **Shen Q, Wang YT, Tian H, Guo FQ.** 2013. Nitric oxide mediates cytokinin functions in cell
 453 proliferation and meristem maintenance in Arabidopsis. *Molecular Plant* **6**, 1214–1225.

454 **Sloan J, Hakenjos JP, Gebert M, Ermakova O, Gumiero A, Stier G, Wild K, Sinning I, Lohmann JU.**
 455 2020. Structural basis for the complex DNA binding behavior of the plant stem cell regulator
 456 WUSCHEL. *Nature Communications* **11**.

457 **Su YH, Zhou C, Li YJ, Yu Y, Tang LP, Zhang WJ, Yao WJ, Huang R, Laux T, Zhang XS.** 2020. Integration
 458 of pluripotency pathways regulates stem cell maintenance in the *Arabidopsis* shoot meristem.
 459 *Proceedings of the National Academy of Sciences*, 202015248.

460 **Sun B, Ito T.** 2015. Regulation of floral stem cell termination in Arabidopsis. *Frontiers in Plant Science*
 461 **6**, 17.

462 **Vernoux T, Brunoud G, Farcot E, et al.** 2011. The auxin signalling network translates dynamic input
 463 into robust patterning at the shoot apex. *Molecular Systems Biology* **7**, 508.

464 **Vidal EA, Alvarez JM, Araus V, et al.** 2020. Nitrate 2020: Thirty years from transport to signaling
 465 networks. *The Plant Cell*, tpc.00748.2019.

466 **Wany A, Foyer CH, Gupta KJ.** 2018. Nitrate, NO and ROS Signaling in Stem Cell Homeostasis. *Trends*
 467 *in Plant Science* **23**, 1041–1044.

468 **Weits DA, Kunkowska AB, Kamps NCW, et al.** 2019. An apical hypoxic niche sets the pace of shoot
 469 meristem activity. *Nature* **569**, 714–717.

470 **Wenkel S, Emery J, Hou BH, Evans MMS, Barton MK.** 2007. A feedback regulatory module formed by
 471 Little Zipper and HD-ZIPIII genes. *Plant Cell* **19**, 3379–3390.

472 **Winter N, Kollwig G, Zhang S, Kragler F.** 2007. MPB2C, a Microtubule-Associated Protein, Regulates
 473 Non-Cell-Autonomy of the Homeodomain Protein KNOTTED1. *The Plant Cell* **19**, 3001–3018.

474 **Wu H, Qu X, Dong Z, et al.** 2020. WUSCHEL triggers innate antiviral immunity in plant stem cells.
 475 *Science* **370**, 227–231.

476 **Yadav RK, Girke T, Pasala S, Xie M, Reddy GV.** 2009. Gene expression map of the Arabidopsis shoot
 477 apical meristem stem cell niche. *Proceedings of the National Academy of Sciences* **106**, 4941–4946.

478 **Yadav RK, Perales M, Gruel J, Girke T, Jonsson H, Reddy GV.** 2011. WUSCHEL protein movement
 479 mediates stem cell homeostasis in the Arabidopsis shoot apex. *Genes & Development* **25**, 2025–
 480 2030.

481 **Yadav RK, Perales M, Gruel J, Ohno C, Heisler M, Girke T, Jönsson H, Reddy GV.** 2013. Plant stem
 482 cell maintenance involves direct transcriptional repression of differentiation program. *Molecular*
 483 *Systems Biology* **9**, 654.

484 **Yoshida S, Mandel T, Kuhlemeier C.** 2011. Stem cell activation by light guides plant organogenesis.
 485 *Genes and Development* **25**, 1439–1450.

486 **Zeng J, Dong Z, Wu H, Tian Z, Zhao Z.** 2017. Redox regulation of plant stem cell fate. *The EMBO*
487 *Journal* **36**, 2844–2855.

488 **Zhang Z, Hu B, Chu C.** 2020. Towards understanding the hierarchical nitrogen signalling network in
489 plants. *Current Opinion in Plant Biology* **55**, 60–65.

490 **Zhao Z, Andersen SU, Ljung K, Dolezal K, Miotk A, Schultheiss SJ, Lohmann JU.** 2010. Hormonal
491 control of the shoot stem-cell niche. *Nature* **465**, 1089–1092.

492 **Zhou Y, Liu X, Engstrom EM, Nimchuk ZL, Pruneda-Paz JL, Tarr PT, Yan A, Kay SA, Meyerowitz EM.**
493 2015. Control of plant stem cell function by conserved interacting transcriptional regulators. *Nature*
494 **517**, 377–380.

495 **Zhou Y, Yan A, Han H, Li T, Geng Y, Liu X, Meyerowitz EM.** 2018. HAIRY MERISTEM with WUSCHEL
496 confines CLAVATA3 expression to the outer apical meristem layers. *Science* **361**, 502–506.

497

498