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EXPERT VIEW

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

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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 CLAVATA3 (CLV3). WUS is a homeodomain transcription factor expressed in the organizing centre that moves to the central zone to promote stem cell fate. CLV3 is a peptide whose expression is induced by WUS in the central zone and that can move back to the organizing centre to inhibit *WUS* expression. Within the past 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 highlight the most recent advances in our comprehension of the molecular mechanisms of WUS function, of its interaction with other transcription factors and 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: Auxin, CLAVATA, cytokinins, light, nitrate, oxygen, shoot apical meristem, WUSCHEL.

Introduction

Plants generate organs throughout their life thanks to the maintenance of stem cell niches localized in specialized tissues referred to as meristems. The shoot apical meristem (SAM) is a highly organized structure that is responsible for the generation of all of the aerial organs of the plant (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 grow and divide relatively slowly and are marked by the expression of the *CLAVATA3* (*CLV3*) gene (Fletcher, 1999). The organizing centre (OC) is located below the CZ at the tip of the rib meristem and is defined by the expression of the stem cell regulator *WUSCHEL* (*WUS*) (Mayer *et al.*, 1998). Cells that are advected away from the CZ 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 is also organized into 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 (Brand, 2000; Schoof *et al.*, 2000) (Fig. 1). WUS is a mobile homeodomain TF 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 have been isolated including CLV1, CLV2, CORYNE (CRN) co-receptors, members of the BARELY ANY MERISTEM (BAM) family, and the recently characterized members of the CLAVATA3 INSENSITIVE RECEPTOR KINASE (CIK) family (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 heterodimers. 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 MITOGEN ACTIVATED PROTEIN KINASE 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 of complexity to the core CLV–WUS feedback loop (Busch *et al.*, 2010). CLV signalling also affects auxin-mediated growth in floral primordia, notably in response to cold (Jones *et al.*, 2020).

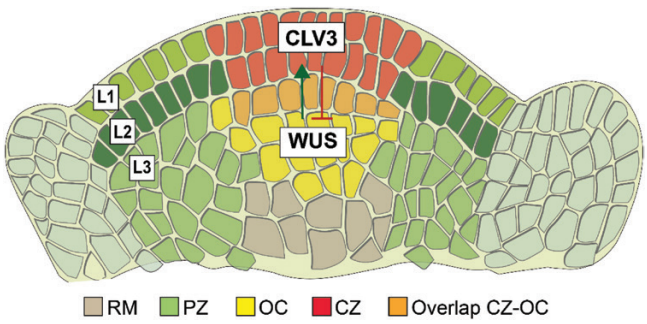


Fig. 1. Organization of the Arabidopsis shoot apical meristem. CZ, central zone; L, layer; OC, organizing centre; PZ, peripheral zone; RM, rib meristem.

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 to characterize the molecular mechanisms of WUS function, its connection to hormonal signalling, and its response to environmental cues in the model plant 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 reporters fused to various florescent proteins, Yadav *et al.* (2011) 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. Following this work, Daum *et al.* (2014) 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. It is probable that the movement of WUS through plasmodesmata is regulated by specific yet uncharacterized proteins localized at the plasmodesmata, similarly to what has been observed for STM (Winter *et al.*, 2007).

Like other homeodomain TFs, 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 *et al.* (2016) identified two distinct regions that are necessary for WUS dimerization. They also proposed that the dimerization could be promoted by the binding to DNA, although this 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 as one of the sequences 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 TFs, 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

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

- **WUS dimerization could explain its dual functions in the organizing centre and in the central zone**

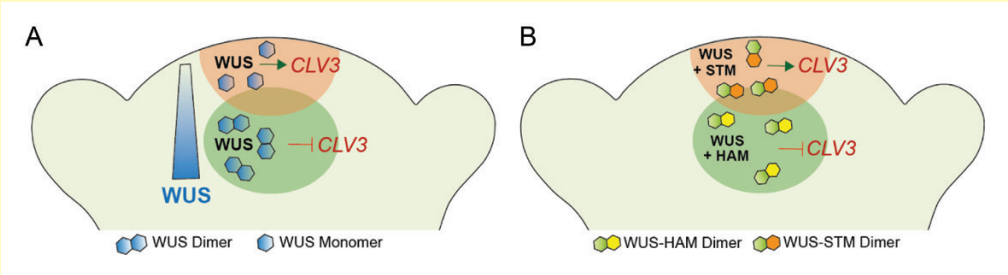
Rodriguez *et al.* (2016) and Perales *et al.* (2016) showed that WUS forms stable homodimers upon binding to DNA in a concentration-dependent manner, which affects its binding to the *CLV3* promoter. They proposed a model in which WUS dimers negatively regulate *CLV3* expression in the OC while WUS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

- **WUS can form heterodimers with HAM in the organizing centre**

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

- **WUS can form heterodimers with STM in the central zone**

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 (B).



et al. (2016) showed that the formation of homodimers could affect both the binding to DNA and the activity of WUS (Box 1). They proposed a model in which WUS monomers would activate *CLV3* in the CZ but WUS dimers would repress *CLV3* in the OC. Sloan and colleagues recently obtained the structure of fragments of WUS proteins bound to different DNA sequences that 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 TFs (Zhou *et al.*, 2015, 2018) (Box 1). The triple *ham1.2.3* mutant has a very intriguing phenotype in which *CLV3* expression increases and moves from the CZ to the OC. Given that HAM is only expressed in L3, Zhou *et al.* (2018) proposed that the formation of heterodimers between WUS and HAM prevents the induction of *CLV3* in the OC while the absence of HAM in L1 and L2 allows the induction of *CLV3* by WUS in the CZ. 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 *et al.* (2020) 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 binding of WUS through the formation of a WUS–STM heterodimer (Box 1).

Taking these studies together, we can build a model in which WUS homodimers and/or WUS–HAM heterodimers would inhibit *CLV3* expression in the OC, while WUS-STM heterodimers or WUS monomers 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 in which WUS activates CK signalling by repressing negative regulators of CK signalling of the type-A *ARABIDOPSIS RESPONSE REGULATOR* (*ARR*) 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 ARABIDOPSIS HISTIDINE KINASE (AHK) cytokinin receptors (Gordon *et al.*, 2009; Chickarmane *et al.*, 2012). Gruel *et al.* (2016) 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 to an unknown molecule restricting expression of AHKs 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 the *WUS* expression domain can scale to the size and the curvature of the SAM (Box 2). Identifying the molecule controlling expression of AHKs should allow testing of the predictions 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 positions in the PZ to induce organ emergence thanks to polar transport mediated by PIN-FORMED 1 (PIN1) (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 *et al.* (2019) proposed that the maintenance of the stem cells in such a low auxin signalling state is controlled by WUS (Box 2). WUS notably reduces the expression of 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 from differentiating. Interestingly, Galvan-Ampudia *et al.* (2020) 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 at the boundaries of the CZ where the temporal integration of auxin concentration allows the activation of auxin signalling (Box 2). From these studies, we can build a model in which 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 feed back 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, the *drn/drnl* double mutant does not recapitulate the *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 levels, sugar levels, mineral nutrient availability, and 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 at several stages of development (Yoshida *et al.*, 2011; Pfeiffer *et al.*, 2016). Accordingly, Pfeiffer *et al.* (2016) demonstrated that light and metabolic signals converge towards the TARGET OF RAPAMYCIN (TOR) pathway 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 (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 *et al.* (2017) 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. Landrein *et al.* (2018) 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 (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

Box 2. Recent developments in understanding the mechanisms of WUS regulation of meristem function

- **WUS modulate auxin signalling in the central zone**

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 central zone but only emerge in the peripheral zone**

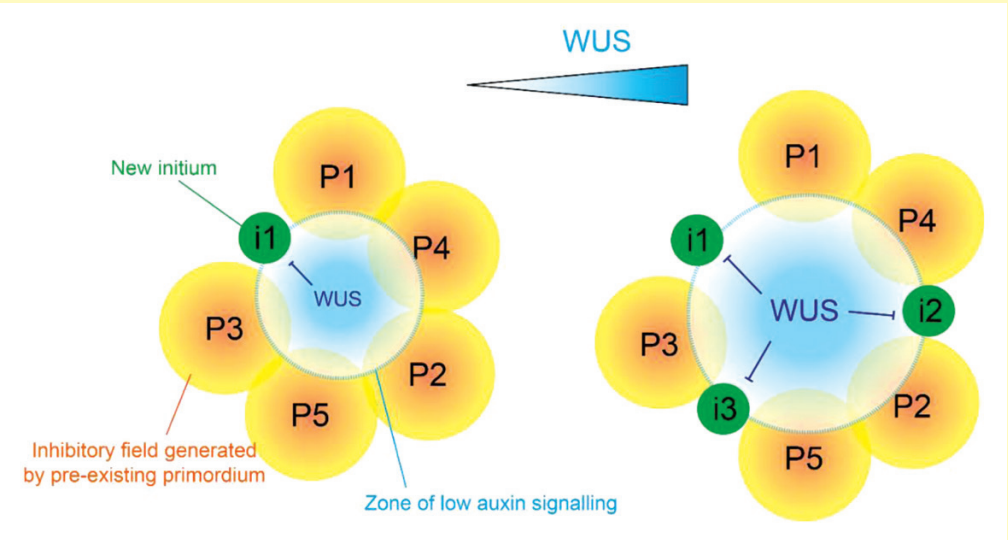
Galvan-Ampudia *et al.* (2020) showed that newly formed auxin maxima corresponding to the future primordia are formed as protrusions originated from the CZ but only emerge in the PZ, which could result from the inhibition of auxin signalling by WUS in the CZ.

- **Auxin can feed back 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*, 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 in their vicinity (by depleting auxin from the surroundings). Interestingly, theoretical work testing this theory and partly validating it 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 *et al.* (2020) and Galvan-Ampudia et al. (2020), we can hypothesize that WUS may control this second parameter by inhibiting auxin signalling. In such a scenario, increasing *WUS* activity would inhibit auxin signalling locally but increase organogenesis globally (see below). This model, which 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 centre 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 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 (A).

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

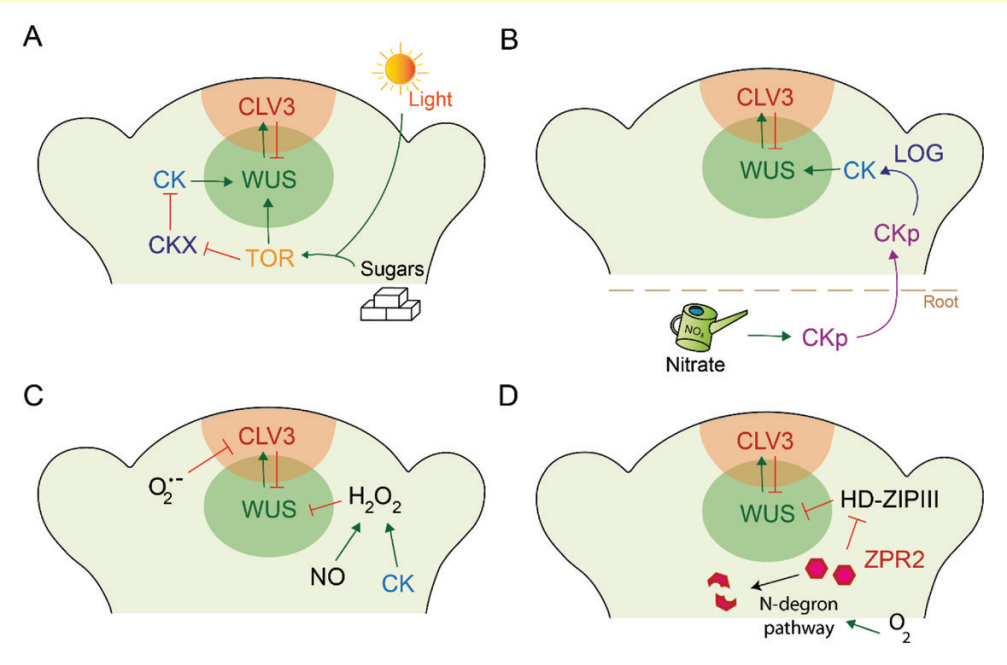
Landrein *et al.* (2018) showed that the SAM can respond to quick changes in nitrate availibility in the soil thanks to long range signalling of CK precursors, which are activated in the SAM and trigger the induction of WUS expression (B).

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

Zeng *et al.* (2017) showed that ROS-metabolizing enzymes display 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 (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-ZIPIII transcription factors. This effect is mediated through the degradation of ZPR2 by the N-degron pathway (D).



oxygen species (ROS), such as NO, superoxide anion ($O_2^{\cdot -}$), or hydrogen peroxide (H_2O_2), affects growth and development in both shoots and roots and is important for SAM robustness against environmental fluctuations (Foyer *et al.*, 2018). Hence, accumulation of ROS species in the SAM following mutation of *AtFTSH4*, encoding 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 leads to a decrease of *WUS* transcript and protein levels and 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 to 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 plants and animals (Le Gac and Laux, 2019). Weits *et al.* (2019), using a microscale oxygen electrode, showed that the SAM is a closed hypoxic niche (Box 3). Consistent with this, a large number of core hypoxia-induced genes are up-regulated in the SAM when compared with juvenile leaves and their expression is down-regulated 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 O_2 (Wenkel *et al.*, 2007; Weits *et al.*, 2019). Several targets of ZRP2, including HD-ZIPIII TFs and HECATE (HEC), have been shown to be involved in stem cell regulation, notably by modulating *WUS* expression.

Recently, Wu *et al.* (2020) 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 of the meristem. How WUS protein is stabilized upon viral infection is still an open question, but this work very interestingly showed a new role for WUS in protecting stem cells from infections.

Conclusions

Accumulating evidence gathered in recent years has highlighted WUSCHEL as 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 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 the 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 highlights very strong connections between stem cell maintenance and hormone signalling. This work notably allowed redefinition of the function of WUS in controlling organogenesis through the inhibition of differentiation. Finally, the recent characterization of the impact of environmental signals 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.

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