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

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7 Abstract:

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

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

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

25 Introduction:

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

40 More than 20 years ago, the maintenance of the stem cell pool in the SAM of the model plant  
41 *Arabidopsis* was proposed to be controlled by a feedback loop between *WUS* and *CLV3* (Schoof *et al.*,  
42 2000; Brand, 2000) (Fig. 1). *WUS* is a mobile homeodomain transcription factor expressed in the OC  
43 that can move to the CZ to promote stem cell fate, notably by repressing differentiation (Yadav *et al.*,  
44 2011; Daum *et al.*, 2014). Plants lacking *WUS* expression are unable to maintain their stem cell niche  
45 in the SAM, which leads to termination of the meristem after the production of a very limited  
46 number of organs (Laux *et al.*, 1996). *CLV3* is a small peptide whose expression is induced by *WUS* in  
47 the CZ but that can repress *WUS* expression (Brand *et al.*, 2002). Plants lacking *CLV3* expression  
48 generate very large meristems producing many organs because of a lack of inhibition of *WUS*  
49 expression (Clark *et al.*, 1995; Fletcher, 1999). Several *CLV3* receptors including *CLV1*, *CLV2*, *CRN*  
50 (*CORYNE*) co-receptors, members of the *BAM* (*BARELY ANY MERISTEM*) family as well as the  
51 recently-characterized members of the *CIK* (*CLAVATA3 INSENSITIVE RECEPTOR KINASES*) family, have  
52 been isolated (Clark *et al.*, 1993; Jeong *et al.*, 1999; DeYoung *et al.*, 2006; Müller *et al.*, 2008;  
53 Nimchuk *et al.*, 2015; Hu *et al.*, 2018). Expressed in the OC but also in different domains in the SAM,  
54 they act in concert to control *WUS* expression by forming a variety of homo and hetero-dimers. The  
55 binding of *CLV3* to *CLV1* was also shown to trigger the internalization of the receptor (Nimchuk *et al.*,  
56 2011), a mechanism that could explain the buffering effects observed following enhancement of  
57 *CLV3* expression (Müller *et al.*, 2006). As for other receptor kinases, activation of *CLV1* induces a  
58 cascade of *MAP KINASE* (*MITOGEN ACTIVATED PROTEIN*) activation ultimately leading to *WUS*  
59 repression through mechanisms that still need to be finely dissected (Betsuyaku *et al.*, 2011). *WUS*  
60 also represses the expression of *CLV1* through direct binding to its promoter thus adding another  
61 layer complexity to the core *CLV/WUS* feedback loop (Busch *et al.*, 2010). *CLV* signalling also affect  
62 auxin-mediated growth in flora primordia, notably in response to cold (Jones *et al.*, 2020).

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

## 70 Molecular mechanisms of *WUS* function

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

82 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  
83 in more depth (Box 1). Rodriguez and colleagues identified two distinct regions that are necessary for  
84 WUS dimerization. They also propose that the dimerization could be promoted by the binding to  
85 DNA, although it may not be strictly necessary (Busch *et al.*, 2010), and that it affects the stability of  
86 the TF (Rodriguez *et al.*, 2016). A further study from the same team showed that the  
87 homodimerization occurs in a concentration-dependent manner and that it affects binding to target  
88 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  
89 (Rodriguez *et al.*, 2016), the formation of dimers may reduce WUS mobility (Fuchs and Lohmann,  
90 2020)(Box 1).

93 WUS has been shown to bind to DNA through three different motifs: a canonical TAAT motif for  
94 homeodomain TF, a G-box like domain and a TGAA domain (Lohmann *et al.*, 2001; Yadav *et al.*, 2011;  
95 Perales *et al.*, 2016). Upon binding, WUS can act as an activator but also as a repressor thanks to the  
96 recruitment of co-repressors from the TOPLESS family (Leibfried *et al.*, 2005; Busch *et al.*, 2010).  
97 Interestingly, Perales and colleagues showed that the formation of homodimers could affect both the  
98 binding to DNA and the activity of WUS (Box 1). They proposed a model where WUS monomers  
99 would activate *CLV3* in the CZ but where WUS dimers would repress *CLV3* in the OC (Perales *et al.*,  
100 2016). Sloan and colleagues recently obtained the structure of fragments of WUS proteins bound to  
101 different DNA sequences which allowed them to study its binding specificity further (Sloan *et al.*,  
102 2020). They showed that WUS preferentially binds to TGAA sequences and that the dimerization  
103 allows cooperative and stabilized binding to specific repeated motifs.

104 In addition to the formation of homodimers, WUS can also form heterodimers with members of the  
105 HAIRY MERISTEM (HAM) family of transcription factors (Zhou *et al.*, 2015, 2018) (Box 1). The triple  
106 *ham1.2.3* mutant has a very intriguing phenotype where *CLV3* expression increases and moves from  
107 the CZ to the OC. Given that HAM is only expressed in the L3, Zhou and colleagues proposed that the  
108 formation of heterodimers between WUS and HAM prevents the induction of *CLV3* in the OC while  
109 the absence of HAM in the L1 and L2 allows the induction of *CLV3* by WUS in the CZ (Zhou *et al.*,  
110 2018). This model is supported by two sets of computational simulations that could recapitulate both  
111 WT and mutant phenotypes (Zhou *et al.*, 2018; Gruel *et al.*, 2018). Very recently, Su and colleagues  
112 also showed that WUS physically interacts with STM. They also demonstrated that STM could bind to  
113 the *CLV3* promoter and that this binding strengthened the one of WUS through the formation of a  
114 WUS/STM heterodimers (Su *et al.*, 2020) (Box 1).

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

#### 120 WUS interaction with hormone signalling

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

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

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

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

165 *WUS* and the transduction of environmental cues

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

175  
176 Nitrogen (N) is a major mineral nutrient for plants whose availability in the soil affects metabolism,  
177 growth, and developmental processes (Vidal *et al.*, 2020). The perception of N in different organs  
178 and the integration of this information through long-distance signalling are key for coping with  
179 changes in N availability. Although nitrate can act as a signal itself, one of the major long-distance N  
180 signalling pathways is mediated by CK (Zhang *et al.*, 2020) (box 3). SAM activity also responds to  
181 changes in nitrate availability in a cytokinin-dependent manner. Osugi and colleagues indeed showed  
182 that an increase in nitrate levels in the soil leads to the production of CK precursors by specific  
183 ISOPENYNYL TRANSFERASE (IPT) enzymes in the root and to their translocation *via* the xylem to the  
184 shoot (Osugi *et al.*, 2017). Landrein and colleagues further showed that the generation of active  
185 cytokinin by LONELY GUY (LOG) enzymes from these precursors in the SAM induces CK signalling, and  
186 WUS expression. This activation leads to stem cell proliferation and increased meristem size and  
187 organ production rate (Landrein *et al.*, 2018) (box 3).

188  
189 However, the effect of nitrate on meristem function may be more complex and only partly mediated  
190 by CK signalling. Nitrate is also a major source of nitric oxide (NO), a central redox signalling molecule  
191 (Fancy *et al.*, 2017). Interestingly, keeping the balance between the different forms of reactive  
192 oxygen species (ROS), such as NO, superoxide anion ( $O_2^{\cdot-}$ ) or hydrogen peroxide ( $H_2O_2$ ), affects  
193 growth and development both in shoots and roots and is important for SAM robustness against  
194 environmental fluctuations (Foyer *et al.*, 2018). Hence, accumulating ROS species in the SAM by  
195 mutating the AtFTSH4, an ATP-dependent mitochondrial protease that counteracts accumulation of  
196 internal oxidative stress, causes meristem termination at higher temperatures (Dolzblasz *et al.*,  
197 2016). Interestingly, the key enzymes regulating ROS metabolism have a distinct spatial distribution  
198 within the SAM and the different forms of ROS play distinct roles in each domain (Yadav *et al.*, 2009;  
199 Zeng *et al.*, 2017; Foyer *et al.*, 2018) (box 3). For example, depleting  $O_2^{\cdot-}$  in the CZ lead to a decrease  
200 of WUS transcript and protein levels, CLV3 expression leading to meristem termination (Zeng *et al.*,  
201 2017).  $H_2O_2$ , on the other hand, is mainly present in the PZ, where it inhibits WUS expression and  
202 promotes stem cell differentiation (Zeng *et al.*, 2017).  $H_2O_2$  production can be promoted via nitrate-  
203 or cytokinin-induced NO, through the induction of superoxide dismutase, which converts superoxide  
204 ( $O_2^{\cdot-}$ ) into  $H_2O_2$ , pointing for a possible role of NO in the regulation of stem cell activity (Wany *et al.*,  
205 2018). Supporting this idea, cytokinins are required for the activation of the *CYCD3* cell cycle gene by  
206 NO, thereby promoting cell proliferation (Shen *et al.*, 2013).

207  
208 Oxygen levels may also affect stem cell homeostasis in both plant and animals (Le Gac and Laux,  
209 2019). Weits and colleagues showed that the SAM is a closed hypoxic niche using a microscale  
210 oxygen electrode (Weits *et al.*, 2019) (box 3). Consistent with this, a large number of core hypoxia-

211 induced genes are upregulated in the SAM when compared to juvenile leaves and their expression is  
212 downregulated when meristems are exposed to higher oxygen concentrations (80%) (Weits et al.,  
213 2019). Most importantly, increasing the levels of oxygen resulted in decreased leaf production rates,  
214 supporting the importance of hypoxia for SAM activity (Weits et al., 2019). Part of this response is  
215 mediated by LITTLE ZIPPER2 (ZPR2), which is degraded by the oxygen-dependent N-degron pathway  
216 and is thus stabilized at low O<sub>2</sub> (Wenkel et al., 2007; Weits et al., 2019). Several targets of ZRP2,  
217 including HD-ZIPIII transcription factors and HECATE (HEC), have been shown to be involved in stem  
218 cell regulation, notably by modulating *WUS* expression.

219

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

## 227 Conclusion

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

243

## 244 Acknowledgments

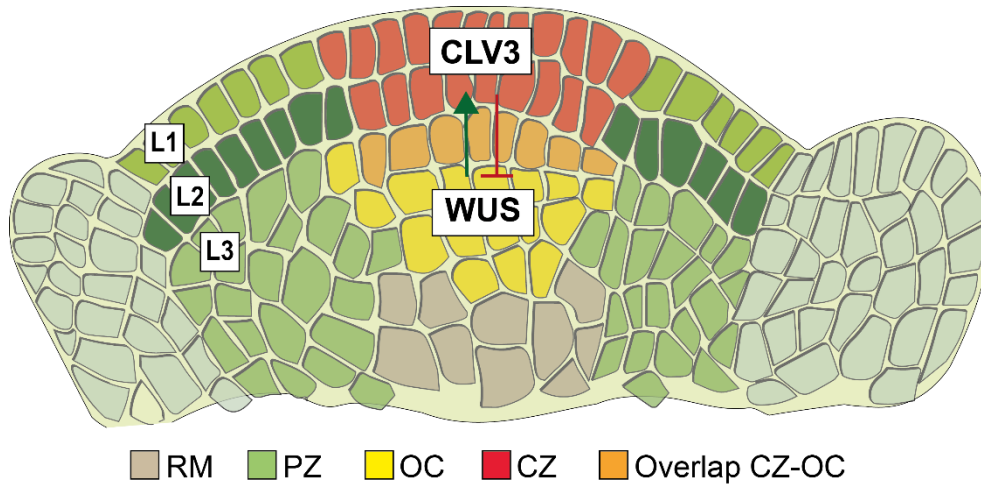
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250

251

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

253 RM: Rib meristem, PZ: Peripheral zone, OC: Organizing centre, CZ: central zone. L1 to L3: Layer 1 to  
 254 layer 3.  
 255



256  
 257  
 258

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

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

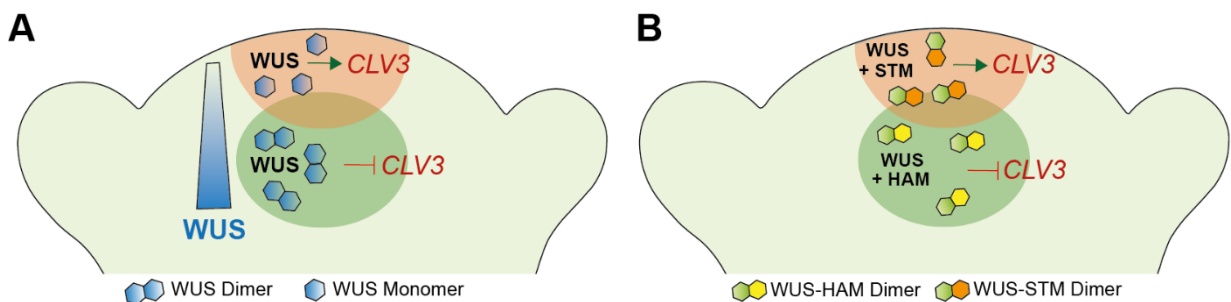
261 Rodriguez *et al.* (2016) and Perales *et al.* (2016) showed that *WUS* forms stable homodimers upon  
 262 binding to DNA in a concentration-dependent, which affects its binding to the *CLV3* promoter. They  
 263 proposed a model where *WUS* dimers negatively regulate *CLV3* expression in the OC while *WUS*  
 264 monomers positively regulate *CLV3* expression in the CZ (panel A).  
 265

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

267 Zhou *et al.* (2018) showed that *WUS* can physically interact with members of the *HAM* family of TF,  
 268 which are specifically expressed in the L3 of the SAM. They proposed a model where *WUS*/*HAM*  
 269 heterodimers repress *CLV3* expression in the OC while *WUS* alone in the CZ induces *CLV3* expression  
 270 (panel B).  
 271

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

273 Su *et al.* (2020) showed that *WUS* can physically interact with *STM* and that *STM* binding to *CLV3*  
 274 promoter can enhance the stability of *WUS* binding to this promoter through the formation of  
 275 heterodimer in the CZ (panel B).  
 276



277



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

280 • **WUS modulate auxin signalling in the CZ**

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

284

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

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

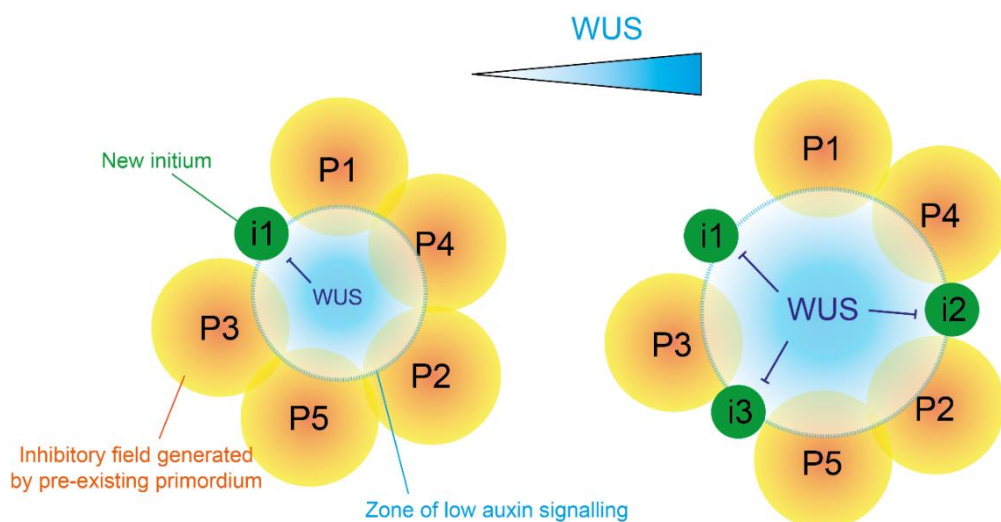
289 • **Auxin can feedback on stem cell regulation through MP/ARF5**

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

293

294 • **A model for WUS control of organogenesis in the SAM**

295 The emergence of new organs in the SAM results from the accumulation of auxin at specific location  
296 thanks to polar auxin transport by PIN1 proteins (Reinhardt *et al.*, 2000). This mechanism of organ  
297 positioning (phyllotaxis) has long been conceptualized by the so-called inhibitory field theory  
298 (Landrein and Vernoux, 2014). This theory states that pre-existing organs inhibit the initiation of new  
299 organs at their vicinity (by depleting auxin from the surroundings). Interestingly, theoretical work  
300 testing this theory and partly validated experimentally have shown that two key parameters could  
301 control the positioning and timing of organ emergence through inhibitory fields: the size of the  
302 inhibitory fields and the radius of the central ring on which organs are initiated (Douady and Couder,  
303 1996; Landrein *et al.*, 2015). By combining the recent work from Ma and colleagues and from Galvan-  
304 Ampudia and colleagues, we could hypothesize that WUS may control this second parameter by  
305 inhibiting of auxin signalling. In such scenario, increasing *WUS* activity would inhibit auxin signalling  
306 locally but increase organogenesis globally (See bellow). This model, that remains to be tested, would  
307 explain the tight correlation that can be measured between *WUS* expression, meristem size (which is  
308 defined as the distance between the center of the SAM and the organs) and organogenesis rate  
309 (Landrein *et al.*, 2015, 2018).



310

311

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

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

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

317  
 318 • **Nitrate can modulate WUS expression through cytokinins**

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

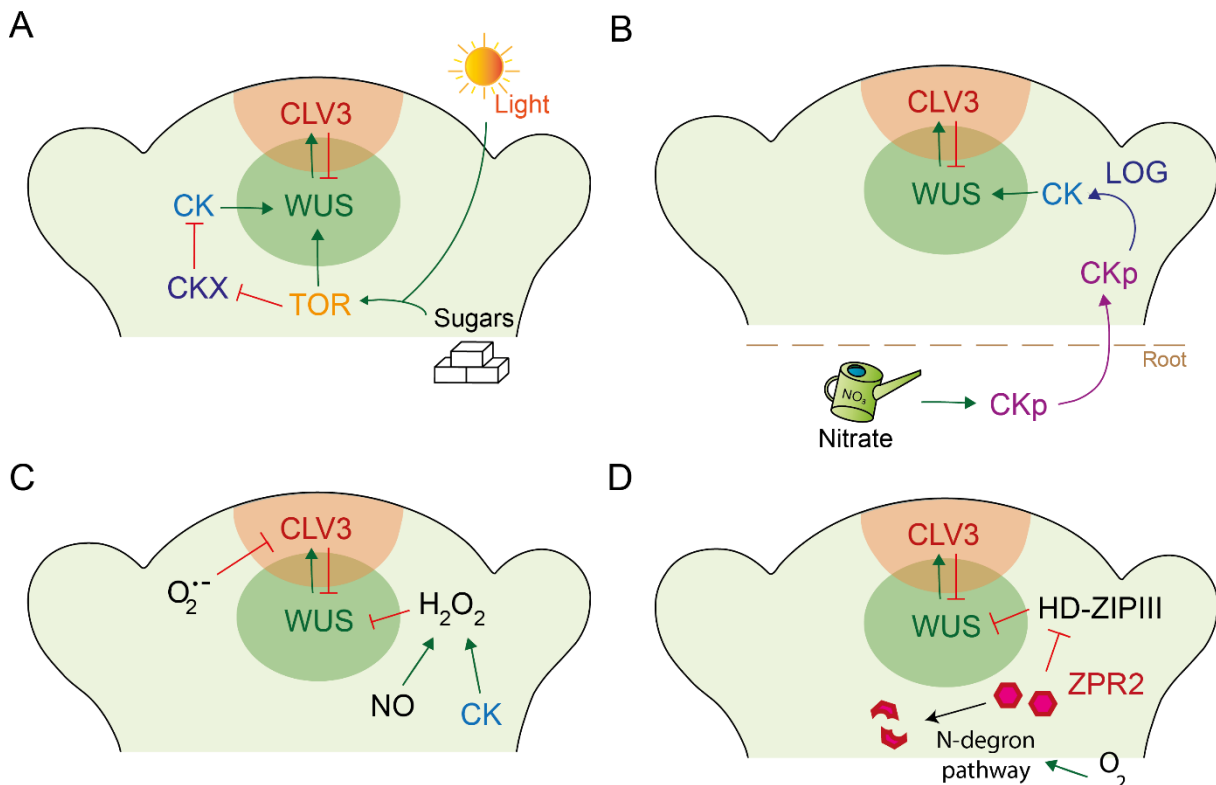
322  
 323 • **ROS can also influence stem cell homeostasis in the SAM**

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

327  
 328 • **Oxygen levels can affect stem cell homeostasis in the SAM**

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

333



334  
 335  
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