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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 ISOPENTENYL 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 (Dolzbłasz *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 O₂ (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

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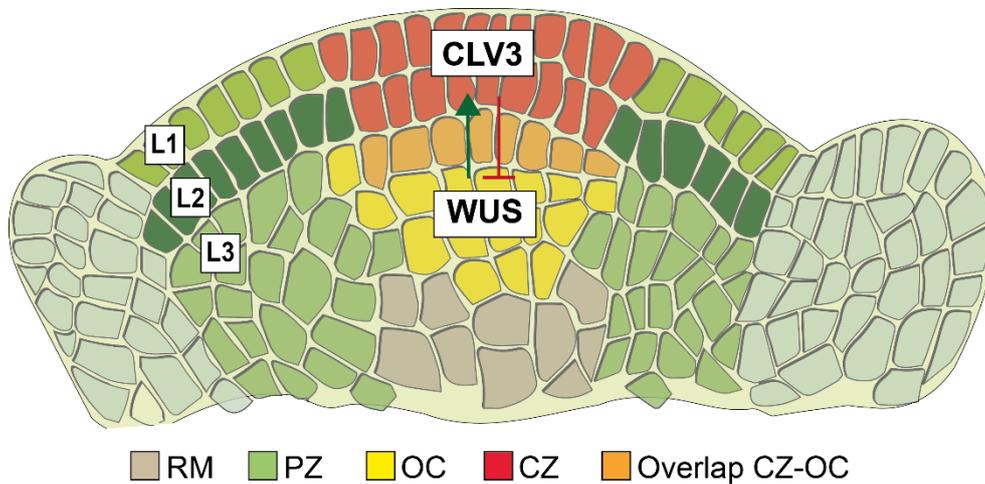
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250

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**

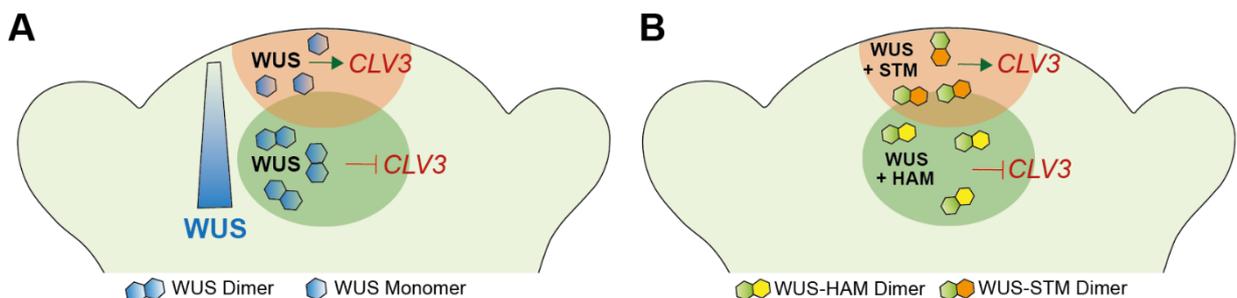
261 Rodriguez *et al.* (2016) and Perales *et al.* (2016) showed that *WUS* forms stable homodimers upon
 262 binding to DNA in a concentration-dependent, which affects its binding to the *CLV3* promoter. They
 263 proposed a model where *WUS* dimers negatively regulate *CLV3* expression in the OC while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

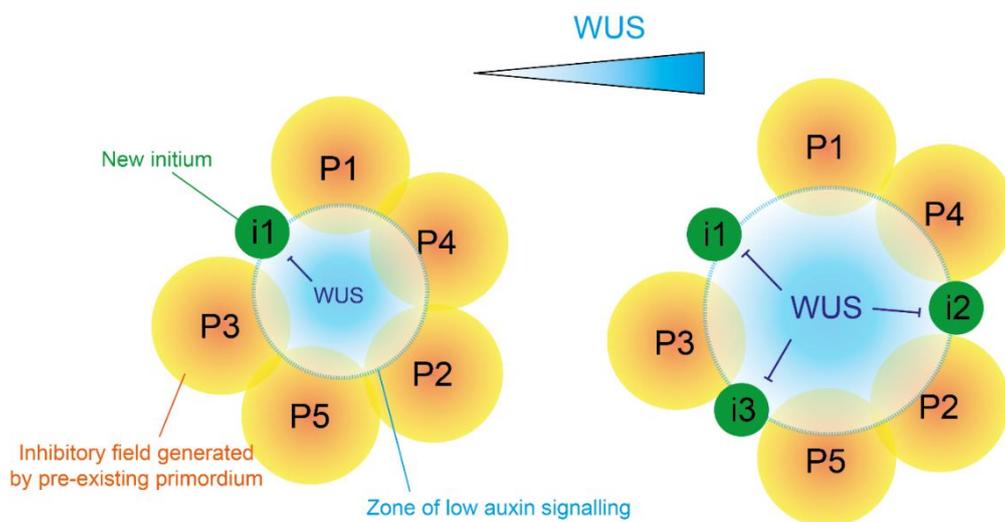
289 • **Auxin can feedback on stem cell regulation through MP/ARF5**

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 below). 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**

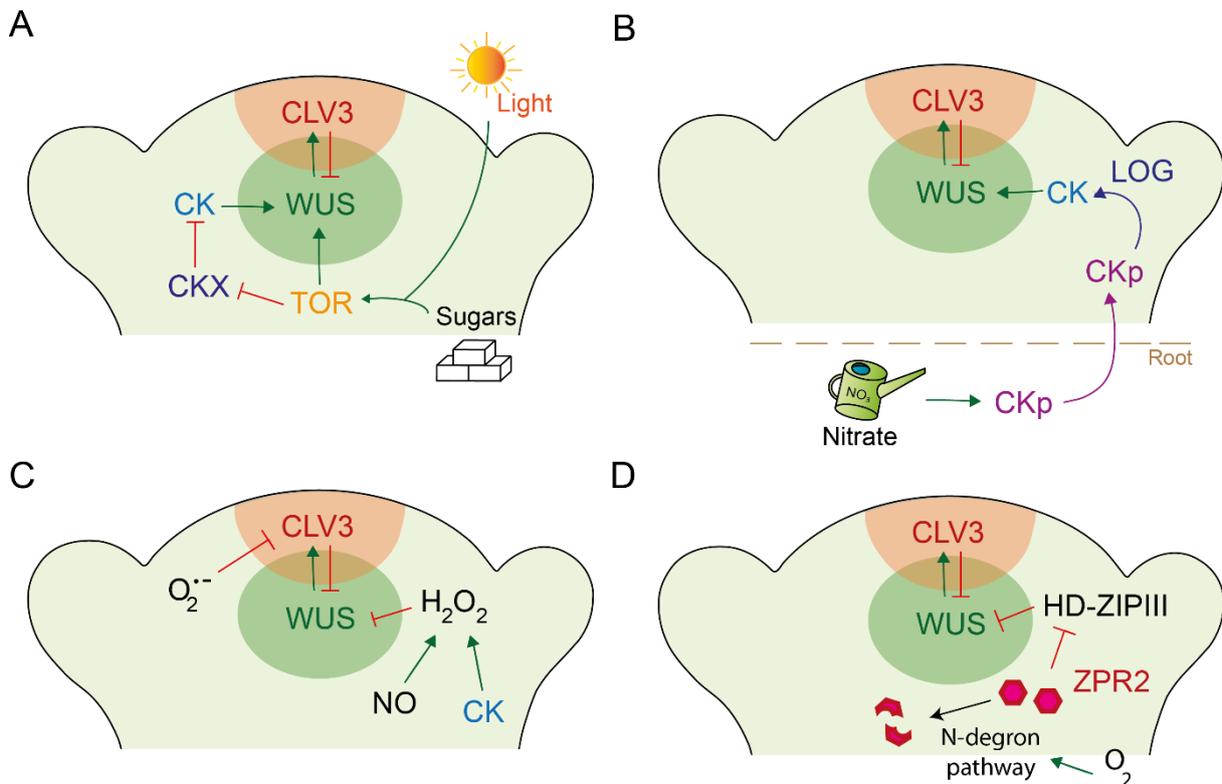
324 Zeng *et al.* (2017) showed that ROS-metabolizing enzymes displays specific patterns of expression in
325 the SAM. They proposed that the balance between $O_2^{\cdot-}$ and H_2O_2 is involved in stem cell
326 maintenance and differentiation in the SAM (panel C).

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).

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