The mutual inhibition between PLETHORAs and ARABIDOPSIS RESPONSE REGULATORs controls root zonation.

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Abstract

During organogenesis a key step towards the development of a functional organ is the separation of cells in specific domains with different activities. Mutual inhibition of gene expression has been shown to be sufficient to establish and maintain these domains during organogenesis of several multicellular organisms. Here we show that the mutual inhibition between the PLTs and the ARRs transcription factors is sufficient to separate cell division and cell differentiation during root organogenesis. In particular, we show that ARR1 suppresses PLTs activities and that PLTs suppress ARR1 and ARR12 by targeting their protein for degradation via the KMD2 F-box protein. These findings reveal new important aspects of the complex process of root zonation and development.

Keywords: root meristem, cell division, cell differentiation

Introduction

Organogenesis is a complex process where tissues and cells at different stages of development need to be coordinated to guarantee the correct shape and function of a given organ. One strategy to organize organ development is the separation of cells into distinct functional domains where specific genes determine identity and activities of the cells. Developmental boundaries are

established to separate these domains and to preserve over time cell function (Wolpert, et al., Principles of Development).

The *Arabidopsis thaliana* root organ at the end of root organogenesis, at 5 days post seed germination (dpg), consists of three distinct domains along the longitudinal axis: the stem cell niche from which all the root tissues arise; the division zone (meristem), where the stem cell daughters actively divide and the elongation/differentiation zone, where cells stop dividing, and start to elongate and differentiate (Figure 1A, Dolan et al., 1993; Mambro et al., 2019; Svolacchia et al., 2020). A developmental boundary is positioned between dividing and differentiating cells called the transition zone (TZ, Figure 1A, Svolacchia et al., 2020; Salvi et al., 2020 a). At this root developmental stage, a dynamic equilibrium is reached where cell division, in the stem cell niche and the meristem, is balanced by cell differentiation in the elongation/differentiation zone. This ensures a stable number of meristematic cells, the maintenance of the TZ position, and indeterminate root growth (Dello Ioio et al., 2007; Moubayidin et al., 2010, Svolacchia et al., 2020; Salvi et al., 2020 a).

In previous work, using an interdisciplinary approach combining molecular genetics with computational modeling, we identified a minimal molecular network that dynamically controls the development of the root organ generating different domains of gene expression and cell activities (Salvi et al., 2020 b). This network is based on the activity of the PLETHORA transcription factors (PLT1 and PLT2, Aida et al., 2004; Blilou et al., 2005) and the cytokinin response factors ARABIDOPSIS RESPONSE REGULATORs (ARR12 and ARR1, Dello Ioio et al., 2008; Salvi et al., 2020 b). Their mutual inhibition positions expression of the PLTs in the stem cell niche and in the meristem - where they control cell division (Galinha et al., 2007, Mähönen et al., 2014), - while restricting expression of the ARRs genes to the elongation/differentiation zone - where they control cell differentiation (Dello Ioio et al., 2008; Di Mambro et al., 2017, Salvi et al., 2020). This network is also responsible for generating the TZ developmental boundary at the early stages of root development and maintaining it at the end of meristem development (Salvi et al., 2020 b). In particular, we showed that an ARR12/PLTs network, by generating the TZ at the early stage of root development, is responsible for controlled root meristem expansion while an ARR1/PLTs network maintains the TZ position at later stage of root development setting meristem size (Salvi et al., 2020 b). Although this network can explain many aspects of the dynamic patterning of the root, several important features are still unclear: while a mutual inhibition between PLTs and ARR12 was observed *in vivo*, mutual inhibition between the PLTs and ARR1 was only predicted by a dynamic root computational model. Furthermore, how the PLTs suppress ARRs activity is still unknown. ARR1 and ARR12 protein stability is regulated by the KISS ME DEADLY (KMD) F-BOX family.

KMD F-BOX proteins physically interact with ARR1 and ARR12 promoting their protein ubiquitination and, hence, proteasome dependent degradation (Kim et al., 2012, Kim et al., 2013). The KMDs family consists of four F-BOXes (KMD1-4) that by controlling ARR1 and ARR12 proteins levels control root development and shoot organogenesis (Kim et al., 2013). Here, we show that a mutual inhibition between PLTs and ARR1 exists *in vivo* and that it contributes to establishing meristem size by positioning ARR1 in the elongation/differentiation zone and the PLTs in the meristem and in the stem cell niche. Furthermore, we demonstrate that PLTs, in order to inhibit ARR1 and ARR12 activities, directly controls the KMD2 protein and promote the expression of KMD1and KMD4 proteins (Kim et al., 2013). These data contribute significant knowledge on the complex dynamic patterning processes at the basis of root zonation and development.

Results

ARR1 confines expression of PLTs to position cell division activity in the root.

The computational model of root growth we developed predicts that a mutual inhibition between the *PLT*s and *ARR1* genes should exist to maintain the position of the TZ and to set meristem size at later stage of root development (Salvi et al., 2020 b) (Figure 1A). We thus set to verify whether this mutual inhibition indeed exists *in vivo* and contributes in positioning the *PLT*s and the *ARR1* genes expression domains.

We first examined the effect of ARR1 on *PLT*s monitoring *PLT2* and *PLT1* expressions in the *arr1-3* loss-of-function mutant by qRT-PCR at 5 dpg (end of root organogenesis). Notably the *PLT2* and *PLT1* mRNA levels are increased in the *arr1-3* mutant confirming an inhibitory effect of ARR1 on *PLT2* and *PLT1* expression (Figure 1B). Furthermore, at 5 dpg, PLT2 and PLT1 protein expression domain in the *arr1*-3 mutants expands to the shoot-ward region of the proliferation zone as revealed by the analysis of *pPLT1::PLT1:YFP* and *pPLT2::PLT2:YFP* constructs in this mutant (Figure 1C, D, E, F). Hence ARR1 defines the *PLTs* expression domain thereby contributing to position cell division activities and meristem development. Whereas, as we have previously shown, ARR12 directly binds the *PLT2* promoter to suppress PLTs expression (Salvi et al., 2020 b), the *PLT*s are not direct target of ARR1 (Di Mambro et al., 2017). However upon induction of a *35S::ARR1ΔDDK:GR* construct by dexamethasone treatments (Dex), where ARR1 is constitutively active and independent from cytokinin, expression of the *PLT1* and *PLT2* genes is already suppressed after 3h of Dex treatments, as visualized via qRT PCR experiments (Figure 1G), and before root meristem shrinking (Figure 1H, I, J) . These data suggest that, although indirect, ARR1

dependent PLTs suppression is necessary to control meristem size. ARR1 probably regulates *PLT*s expression acting on the levels and distribution of auxin (Dello Ioio et al., 2008, Di Mambro et al., 2017), the plant hormone that controls *PLT1* and *PLT2* expressions (Aida et al. 2004, Mähönen et al., 2014).

PLTs confines *ARR1* expression to position cell differentiation activity in the root.

We next asked whether PLTs suppress ARR1 activity as predicted by the model (Salvi et al., 2020 b). *ARR1* is not a direct target of the PLT factors, as revealed by the available ChIP-seq and microarray datasets (Santuari et al., 2016). We first set to gain specific information on the cellular localization of the ARR1 protein generating an ARR1/GREEN FLUORESCENT PROTEIN (GFP) translational fusion *(pARR1::ARR1:GFP* plants*)* under the control of the ARR1 promoter. As previously reported, in wild type (Wt) plants *pARR1::ARR1:GFP* expression is restricted at the TZ of all the root tissues (Dello Ioio et al., 2017, Figure 2A). On the contrary, the fully complementing *pARR1::ARR1:GFP* protein fusion expressed in the *plt1,plt2* double mutant expands into the meristem and the stem cell niche (Figure 2B). The high levels of the ARR1 protein observed are functional since genes directly controlled by ARR1, such as SHORT HYPOCOTYL2 (*SHY2,* Dello Ioio et al., 2008), GRETCHEN HAGEN3.17 (*GH3.17,* Di Mambro et al., 2017*)*, KIP-RELATED PROTEIN2 (*KRP2,* Salvi et al., 2020*)* and EXPANSIN1 (*EXPA1,* Pacifici et al., 2018) were also up-regulated as revealed by Real-Time qPCR experiments (Figure 2C).

These data suggest that a PLTs/ARR1 mutual inhibition exists *in vivo* and that it is important to confine ARR1 at the elongation/differentiation zone to ensure meristem size stabilization upon completion of root organogenesis (5 dpg). As we have previously demonstrated that ARR12 and ARR1 are partially redundant (Dello Ioio et al, 2007), to understand whether expansion of the *ARR1* protein expression in the meristem and in the stem cell niche is responsible for an increased cell differentiation rate and thus for the short root of the *plt1,plt2* double mutant, we analyzed the root meristem of a *plt1,plt2,arr1,arr12* mutant plants. Interestingly, while the root meristem of *plt1,plt2* mutant plants were fully differentiated already at 6 dpg, the root meristem of *plt1,plt2,arr1,arr12* kept on growing to fully differentiate only at later stage of root development (Figure 3A, B, C, D, E). These data indicate that in the *plt1,plt2* mutant, expansion of the *ARR1* (this work) and *ARR12* (Salvi et al., 2020 b) genes expression domain determines an increased cell differentiation rate responsible for the decrease in meristem size.

PLT-dependent degradation of the ARR1 and ARR12 proteins is required to position cell differentiation in the root.

To understand how the PLTs inhibit ARR1 activities we analyzed the available ChIP-seq and microarray datasets (Santuari et al., 2016). We notice that PLT2 directly binds the promoter of the *KMD2* gene, encoding for an F-box protein that targets ARR1 and ARR12 proteins for degradation (Kim et al., 2013). KMD2 together with KMD1 and KMD4 control root meristem development as multiple mutant combinations of *kmd1,2,4* present fewer cells in their root meristems in comparison to Wt due to ARR1 and ARR12 proteins stabilization (Kim et al. 2013). Notably, analysis of roots of plants carrying a *pKMD2::GFP* construct revealed that *KMD2* is expressed within the PLTs expression domain, in the stem cell niche and the lateral root cap (Figure 4 A).

KMD2 is positively controlled by PLT2, as in the *plt1,plt2* double mutant background *KMD2* mRNA are lower compared to the Wt as shown by qRT-PCR analysis (Figure 4C). Furthermore, plant overexpressing a Dex inducible version of *PLT2* (*35S::PLT2:GR* plants) showed higher *KMD2* mRNA levels upon short induction time (Figure 4D). Although not present in the ChIP-seq and microarray datasets (Santuari et al., 2016) we hypothesized that PLT2 also regulate the expression of KMD1 and 4. To test this hypothesis we analyzed the mRNA levels of these genes in *plt1,2* loss of function mutants via qRT-PCR experiments. We noticed a decrease in mRNA levels of both *KMD1* and *KMD4* in the *plt1,plt2* background, suggesting that PLTs indirectly also regulate the expression of these genes (Figure 4E, F). Corroborating this possibility, analysis of the root meristem of plants harboring *pKMD1::GFP* construct revealed that *KMD1* is expressed within the PLTs expression domain, in the quiescent center (QC), the columella stem cells and the vascular tissue (Figure 4B), resembling the expression of PLTs proteins. Thus, KMD proteins could be the target genes through which PLTs modulate ARR1 and ARR12 activities to control meristem development.

PLTs/ARRs mutual inhibition is necessary for the control cell differentiation and cell division in the meristem but not for the stem cell niche.

To assess whether the PLTs control ARR1 protein distribution and thus root development via KMD2, we generated *plt1,plt2* mutant plants expressing the KMD2 protein under the control of the *PLT2* promoter (*plt1,plt2, PLT2::KMD2* plants) and monitored their root meristem development over time. Interestingly, while the root meristem of *plt1,plt2* mutants plants were fully differentiated already at 6 dpg, the root meristem of *plt1,plt2, PLT2::KMD2* plants kept on growing and completely differentiated only at later stage of root development (Figure 5A, B, C, D, E). These results suggest that to control cell division activities in the meristem, PLTs need to confine ARRs to the elongation/differentiation zone via a KMD2 dependent protein degradation process.

Discussion

In multicellular organisms coherent organ growth is often achieved by organizing cells into distinct functional domains that must be kept separated during organogenesis to guarantee the final functional shape of the organ (Wolpert, et al., 2002, Principles of Development).

In several different developmental contexts such as the early *Drosophila* embryo and the vertebrate neural tube, mutual inhibition of transcription factors has been shown to be sufficient to produce distinct and stable domains of gene expression separated by a sharp and stable boundary (Briscoe and Small, 2015).

Combining molecular genetics with computational modeling we have previously shown that mutual inhibition between the PLTs and ARRs transcription factors play a crucial role during root organogenesis in *Arabidopsis thaliana* (Salvi et al., 2020 b).

Here we uncover new crucial features of this network verifying *in vivo* the PLTs /ARR1 mutual inhibiting network and revealing the molecular nature of the inhibiting effects of the PLTs on the ARRs. The PLTs /ARR1 mutual inhibiting network was previously only predicted by a computational model. Here we identify this interaction *in vivo* and demonstrate that it is responsible of positioning PLTs and *ARR1* expression and activities domains in the meristem and in the elongation/differentiation zone respectively, thus stabilizing the position of the TZ and meristem size at late stage of root development. Furthermore, we show that PLTs, by directly activating expression of the F-box *KMD2* gene (Santuari et al., 2016; Kim et al., 2013), leads to degradation of the ARR1 and ARR12 proteins in the meristem thus contributing in sharpening the TZ developmental boundary and positioning cell differentiation activity at the elongation/differentiation zone.

Recently a synthetic gene circuit characterized by mutual inhibition activities downstream of single diffusible morphogens, has been shown to be sufficient to generate mutually exclusive domains of gene expression (Grant et al., 2020). Interestingly, both PLTs and ARRs are responsive to auxin, a well-known putative plant morphogen. Analogously, in the root meristem auxin may act as the morphogen that orchestrates this complex and dynamic developmental pathway leading to root maturation. This would provide an elegant example of how self-organized gene expression domains can be generated in response to a single morphogenetic gradient in plants. However, whether auxin truly is a *bona fide* plant morphogen remains to be proven.

Materials and methods

Plant material and growth conditions

Wt, *arr1-3;arr12-1 (arr1,12)* , *plt1-4; plt2-2 (plt1,2)* mutants are in Ws/Col mixed ecotype. *pPLT1::PLT1:YFP*, *pPLT2::PLT2:YFP*, *35S::ARR1ΔDDK:GR*, *35S::PLT2:GR* transgenic lines are in Col-0 ecotype. *pARR1::ARR1:GFP*, *pKMD2::GFP*, *pKMD1::GFP*, and *pPLT2::KMD2:GFP* were obtained transforming the constructs via floral dip. *arr1-3; pPLT1::PLT1:YFP*, *arr1-3; pPLT2::PLT2:YFP*, *plt1-4; plt2-2;pARR1::ARR1:GFP* were obtained by crosses of homozygotes lines. *Arabidopsis* seeds were surface sterilized with chlorine gas, and seedlings were grown on ½ Murashige and Skoog (MS) medium at 22°C in long-day conditions (16 hours light/ 8 hours dark cycle).

Generation and Characterization of transgenic plants

For generating *pARR1::ARR1:GFP*, *pPLT2::KMD2:GFP*, *pKMD2::GFP* and *pKMD1::GFP* transgenic plants it was used the Gateway molecular cloning protocol as in Di Ruocco et al., 2018 (Invitrogen). For all the lines, the promoter sequences of *PLT2*, *KMD2*, *KMD1*, and *ARR1*, and genomic sequences of *KMD2* and *ARR1* were amplified from genomic DNA of *Arabidopsis thaliana* Col-0 ecotype using the following primers: pPLT2 cloning FW, 5'-GGGGACAACTTTGTATAGAAAAGTTGTGGTTTGGTAAGTTTACTTAC-3'; pPLT2 cloning REV, 5'-GGGGACTGCTTTTTTGTACAAACTTGGCTTTGATTCCAAGAAAAGGGAA-3'; pKMD2 clonong FW, 5'-GGGGACAACTTTGTATAGAAAAGTTGCACCCTTAATGATCTGGTGAATTTAGAACC-5'; pKMD2 cloning RV, 5'-GGGGACTGCTTTTTTGTACAAACTTGTCAGGTTGAGAGAGATATGAATGAG-3'; pKMD1 cloning FW, 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTATGGAACTTATCCCCAATCTTCC-3'; pKMD1 cloning RV, 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCGACCTCCAAGAAGCAGCCA-3'; pARR1 cloning FW, 5'-GGGGACAACTTTGTATAGAAAAGTTGGAGAGGTCGATGCAAATGGT-3'; gKMD2 cloning FW, 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTATGGAGCTTATTCCTGATCTTCCC-3'; gKMD2 cloning RV, 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTTATCTCCAAGAAGCAACCAGCTTG-3'; pARR1 cloning RV, 5'-GGGGACTGCTTTTTTGTACAAACTTGACCTCTCTCTATGTAGCTCGAACC-3'; gARR1 cloning FW, 5'- GGGGACAGCTTTCTTGTACAAAGTGGATGATGAATCCGAGTCACGG -3'; gARR1 cloning RV, 5'-GGGGACAACTTTGTATAATAAAGTTGTCAAACCTGCTTAAGAAGTGCG -3'. The

sequences were cloned in *pDONOR-P4P1* and *pDONOR-221* vectors for the promoter and for the genomic respectively: *pDONOR-P4P1-pPLT2*, *pDONOR-P4P1-pKMD1*, *pDONOR-P4P1-pKMD2*, *pDONOR-P4P1- pARR1*, *pDONOR-221-gKMD2*, and *pDONOR-P2P3-gARR1*. The LR reaction was conducted by using the *pDONOR-P4P1-pPLT2*, the *pDONOR221-pKMD2*, *pDONORP2P3-GFP*, and the *pBm43GW* destination vectors for *pPLT2::KMD2:GFP* transgenic line. For *pKMD1::GFP*, the LR reaction was conducted by using the *pDONOR-P4P1-pKMD1*, the *pDONOR221-ER-GFP, pDONOR-NOST2,* and the *pBm43GW* destination vector. For *pKMD2::ER-GFP*, the LR reaction was conducted by using the *pDONOR-P4P1-pKMD2*, the *pDONOR221-ER-GFP*, *pDONORP2P3-NOST2*, and the *pBm43GW* destination vectors. For the *ARR1::ARR1:GFP*, the reaction was performed by using the *pDONOR-P4P1-pARR1*, the *pDONOR221-GFP*, *pDONORP2P3-gARR1*, and the *pBm43GW* destination vectors.

RNA isolation and qRT-PCR

Total RNA was extracted from 5 dpg old root tissues using the TRIzol reagent (Invitrogen). The first strand cDNA was synthesized using the Superscript® III First Strand Synthesis System (Invitrogen). Quantitative RT-PCR analysis was conducted using the following gene-specific primers: ACTIN-FW qRT-PCR, 5'-ACCAGCTCTTCCATCGAGA-3'; ACTIN-RV qRT-PCR, 5'-CAAACGAGGGCTGGAACAAG-3'; PLT1-FW qRT-PCR, 5'-CCAAAGTGGTAGTGATTTATTGATT-3'; PLT1-RV qRT-PCR, 5'-GAATTCATTTTCTTCTTTTTGAGTC-3'; PLT2-FW qRT-PCR 5'-GCAGCCATACTTGGAGAAA-3'; PLT2-RV qRT-PCR, 5'-TTCTTGGAATCAAAGCTTAAACCA-3'; KMD1-FW qRT-PCR, 5'-AGCTTCCTCCGATTCCTGGTCAAA-3'; KMD1-RV-qRT_PCR, 5'-CACGCGCCATTTGGAAGTGAGAAA-3'; KMD2-FW qRT-PCR, 5'-TGGTGTATGACGTGGCAGAAGACA-3'; KMD2-RV qRT-PCR, 5'-ACCGATGACATGGAATTTGCCAGC-3'; KMD4-FW qRT-PCR, 5'-GCGTTTATAACGCAACGCT-3'; KMD4-RV qRT-PCR, 5'-TCTCCGGCGAAGAAATCCA-3'; SHY2 FW qRT-PCR, 5'-GGGCAAGATCTATGTTCATTGG-3'; SHY2 RV qRT-PCR, 5'-ACCTTTTGCCCTGTTTCTGA-3'; GH3.17 FW qRT-PCR, 5'- CGCTGAAAAGTCGTGGGAAG -3'; GH3.17 RV qRT-PCR, 5'- AGGAAACATCGGCAGGATCA -3'; KRP2 FW qRT-PCR, 5'-CTCCTCCGGTTGAAGAACAG-3'; KRP2 RV qRT-PCR, 5'- TTTCACGATCGTCACCGTTA-3'; EXPA1 FW qRT-PCR 5'-AAGGCTATGGAACCAACACG-3'; EXPA1 RV qRT-PCR, 5'-GTTGTTCGGTAAGGCGTTGT-3'. PCR amplification was carried out in the presence of the double-strand DNA-specific dye SYBR Green (Quantace). Amplification was monitored in real time with the 7300 Real Time PCR System (Applied Biosystems). Amplification of *ACT2* gene was

used as housekeeper control. Experiments were performed three times on two independent RNA batches, and results were comparable in all experiments. Data are expressed in 2-ΔΔCt. Student's t-test was performed to assess the significance of the difference between each sample and the control sample.

DIC and confocal microscopy

DIC microscope with Nomarski technology (Zeiss Axio Imager.A2) was used to root meristem visualization of wild type, *35S::ARR1ΔDDK:GR*, *plt1,plt2*, *pPLT2::KMD2, plt1,plt2;pPLT2::KMD2, arr1,12, arr1,12,plt1,plt2*. Plants were mounted in chloral hydrate solution (8:3:1 mixture of chloral hydrate:water:glycerol). Laser scanning confocal microscopy (Zeiss LSM 780) was used to examine roots of 5 days old plants stained with 10 μM propidium iodide (Sigma) solution to visualize the cell wall.

Data Availability

Accession numbers are: PLT1, AT3G20840; PLT2, AT1G51190; ARR1, AT3G16857; ARR12, AT2G25180; KMD1, AT1G80440; KMD2, AT1G15670; KMD4, AT3G59940; SHY2, AT1G04240; GH3.17, AT1G28130; KRP2, AT3G50630; EXPA1, AT1G69530.

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Disclosures

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Figure Legends

Figure1: ARR1 suppresses PLTs activities. (A) Graphical representation of a root meristem. Expression of the PLTs is marked in light green, ARR1 in yellow, the Stem Cell Niche (SCN) is highlighted in violet, DZ division zone, TZ transition zone, EDZ elongation/differentiation zone. Blunt arrows indicate ARR1 and PLTs mutual inhibition at the TZ developmental boundary. (B) Bar plot showing relative expression of *PLT1* and *PLT2* genes in wild type (Wt) and *arr1-3* mutant plants at 5 days post germination (dpg). N=3, Error bars=SD, *** indicates a significance with a p-value < 0.001, Student's t-test. Confocal images of 5 dpg old root meristem of *pPLT1::PLT1:*YFP (C), *arr1-3; pPLT1::PLT1:YFP* (D), *pPLT2::PLT2:YFP* (E) and, *arr1-3; pPLT2::PLT2:YFP* (F). Scale bar=50 µm; blue arrowheads indicate SCN while white arrowheads mark the TZ. (G) *PLT1* and *PLT2* relative expression in *35S::ARR1DDK:GR* untreated (MS) and treated with dexamethasone (Dex) for 3 hours. N=3, Error bars=SD, *** indicates a significance with a p-value < 0.001, Student's t-test. Root meristems of *35S::ARR1DDK:GR* plants at 5 dpg untreated (H) and treated with dexamethasone (Dex) 5 µM for 3h (I) and 24h (J). Scale bar, 50 µm. Blue arrowheads and white arrowheads indicate the SCN and the TZ, respectively.

Figure 2: PLTs suppress ARR1 activities. Confocal images of 5 dpg root meristem of *pARR1::ARR1:GFP* (A) and *plt1, plt2; pARR1::ARR1:GFP* (B). Scale bars=50 µm. Blue arrowheads indicate SCN; white arrowheads indicate TZ. (C) Bar plot showing relative expression of ARR1 positively regulated genes (*GH3.17, EXPA1, KRP1, SHY2*) in Wt and the *plt1,plt2* double mutant plants at 5 dpg. N=3, n=2. Error bars=SD, *** indicates a significance with a p-value < 0.001, Student's t-test.

Figure 3: PLTs restrict *ARR1* expression. Root meristems of Wt plant (A), *arr1,arr12* mutant plant (B), *plt1,plt2* double mutant (C) and *plt1,plt2,arr1,arr12* quadruple mutant (D) at 6 dpg. Scale bars, 50 µm. Blue arrowheads and white arrowheads indicate the SCN and the TZ, respectively. (E) Root meristem cell number of Wt, *arr1,arr12* mutant plant, *plt1,plt2* double mutant and *plt1,plt2,arr1,arr12* quadruple mutant at 6 dpg . Error bars=SD, *** indicates significance with a p-value < 0.001, Student's t-test.

Figure 4: PLT2 directly activates *KMD1* and *KMD2* expression. Confocal images of 5 dpg old root meristem of *pKMD2::GFP* (A) and *pKMD1::GFP* (B) plants. Scale bars=50 µm; blue arrowheads indicate SCN while white arrowheads mark the TZ. (C) Bar plot showing relative expression of KMD2 gene in Wt and in *plt1,plt2* double mutant plants at 5 dpg. N=3, n=2. Error bars=SD, *** indicates a significance with a p-value < 0.001, Student's t test. (D) Bar plot showing relative expression of *KMD2* gene in *35S::PLT2:GR* plants untreated (MS) and treated with dexamethasone (Dex) N=3,n=2. Error bars=SD, *** indicates a significance with a p-value < 0.001, Student's t-test. (E) Bar plot showing relative expression of *KMD1* gene in Wt and in *plt1,plt2* double mutant plants at 5 dpg. N=3, n=2. Error bars=SD, *** indicates a significance with a p-value < 0.001, Student's t test. (F) Bar plot showing relative expression of *KMD4* gene in Wt and in *plt1,plt2* double mutant plants at 5 dpg. N=3, n=2. Error bars=SD, *** indicates a significance with a p-value < 0.001, Student's t test.

Figure 5: KMD2 dependent ARR1 and ARR12 protein degradation is necessary to control meristem size. Root meristems of Wt (A), *PLT2::KMD2* plant (B), *plt1,plt2* double mutant (C) and *plt1,plt2; PLT2::KMD2* plant (D) at 6 dpg. Scale bars, 50 µm. Blue arrowheads and white arrowheads indicate the SCN and the TZ, respectively. (E) Root meristem cell number of Wt, *PLT2::KMD2* plant, *plt1,plt2* double mutant and *plt1,plt2; PLT2::KMD2* plant at 6 dpg. Error bars=SD, *** indicates significance with a p-value < 0.001, ** indicates a significance with a p-value < 0,05, Student's t-test.

Figure 1:

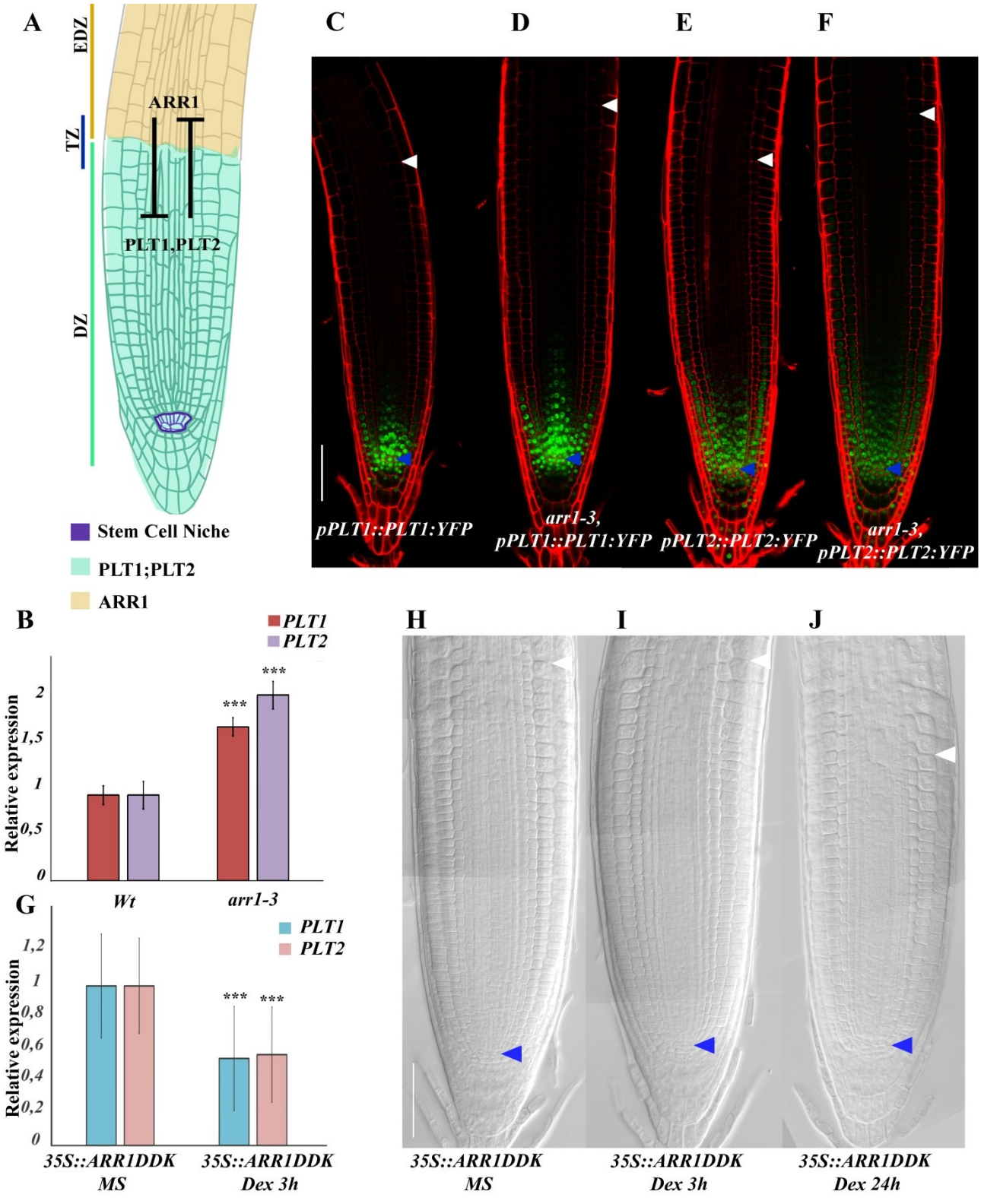
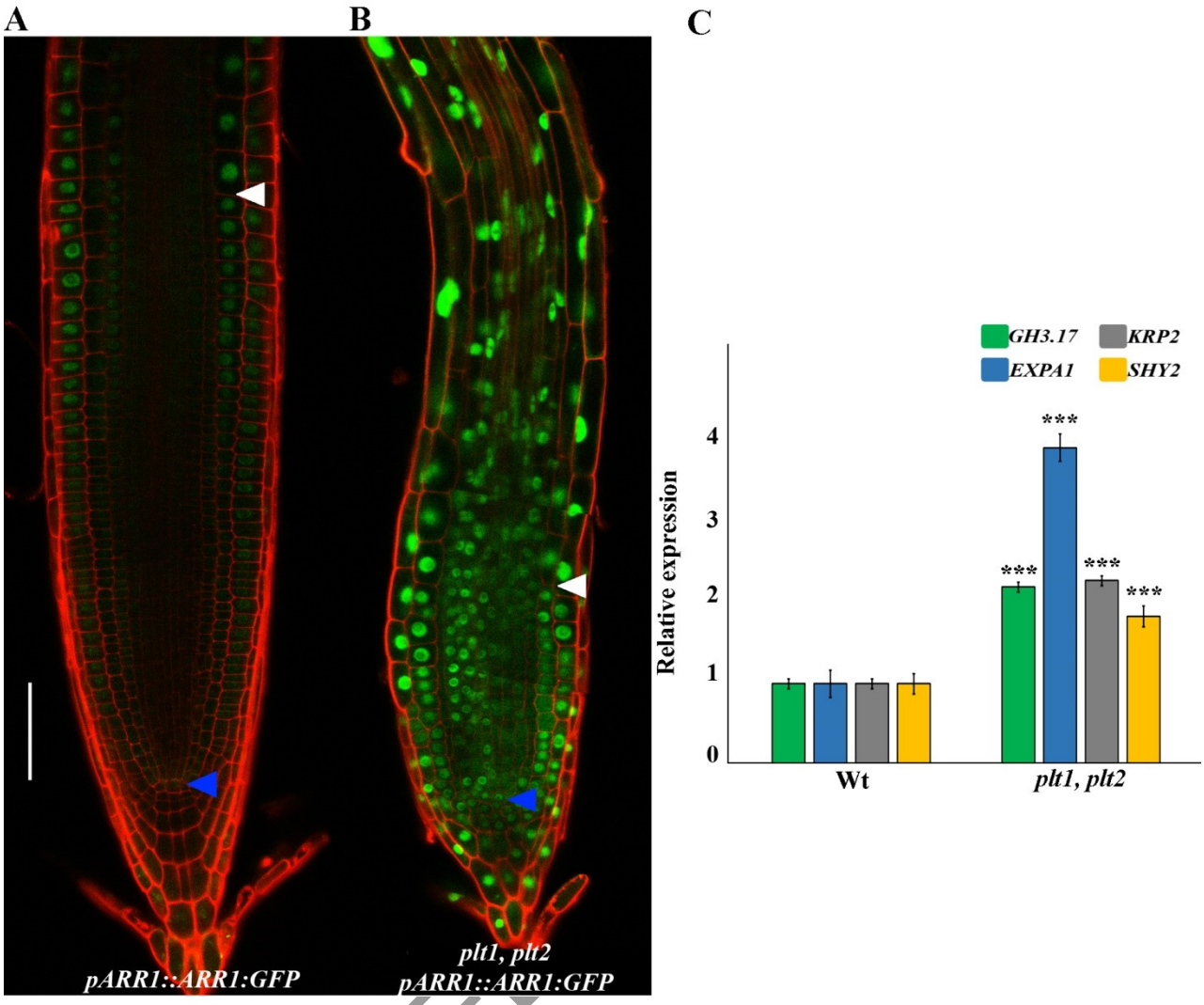


Figure 2:



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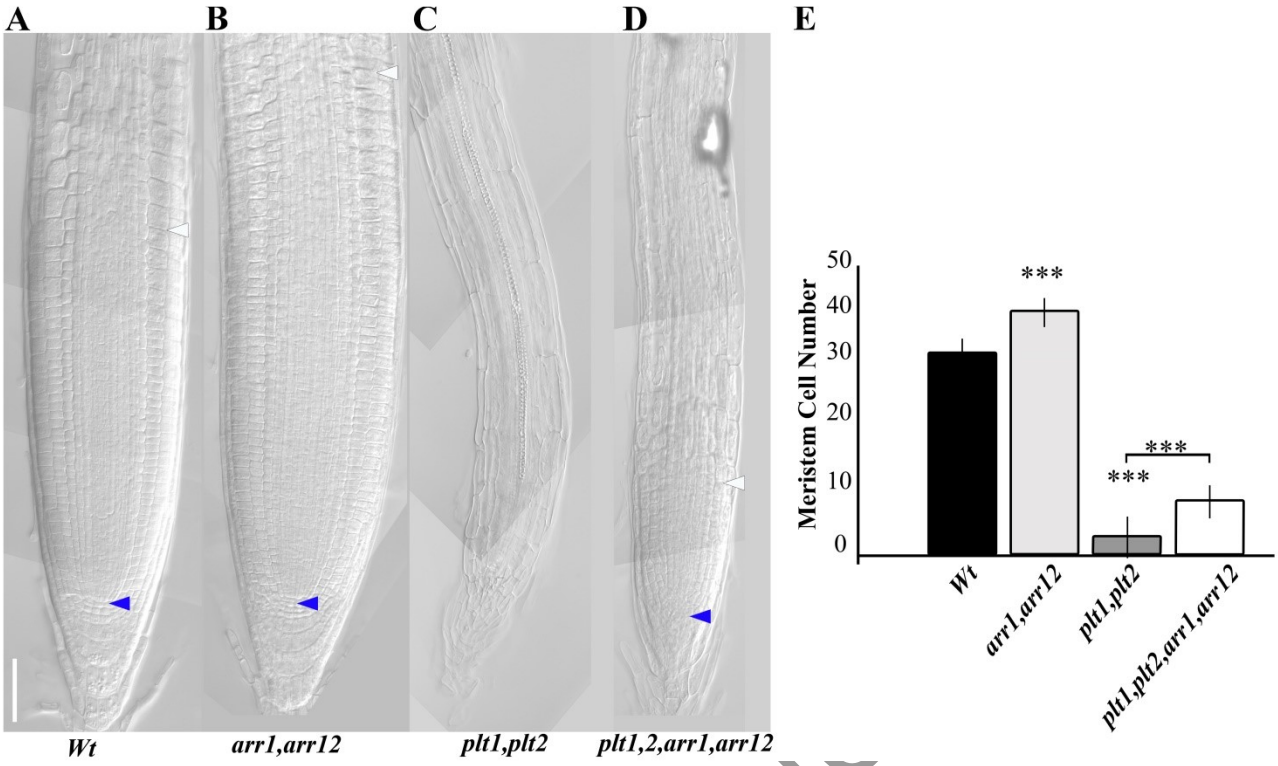

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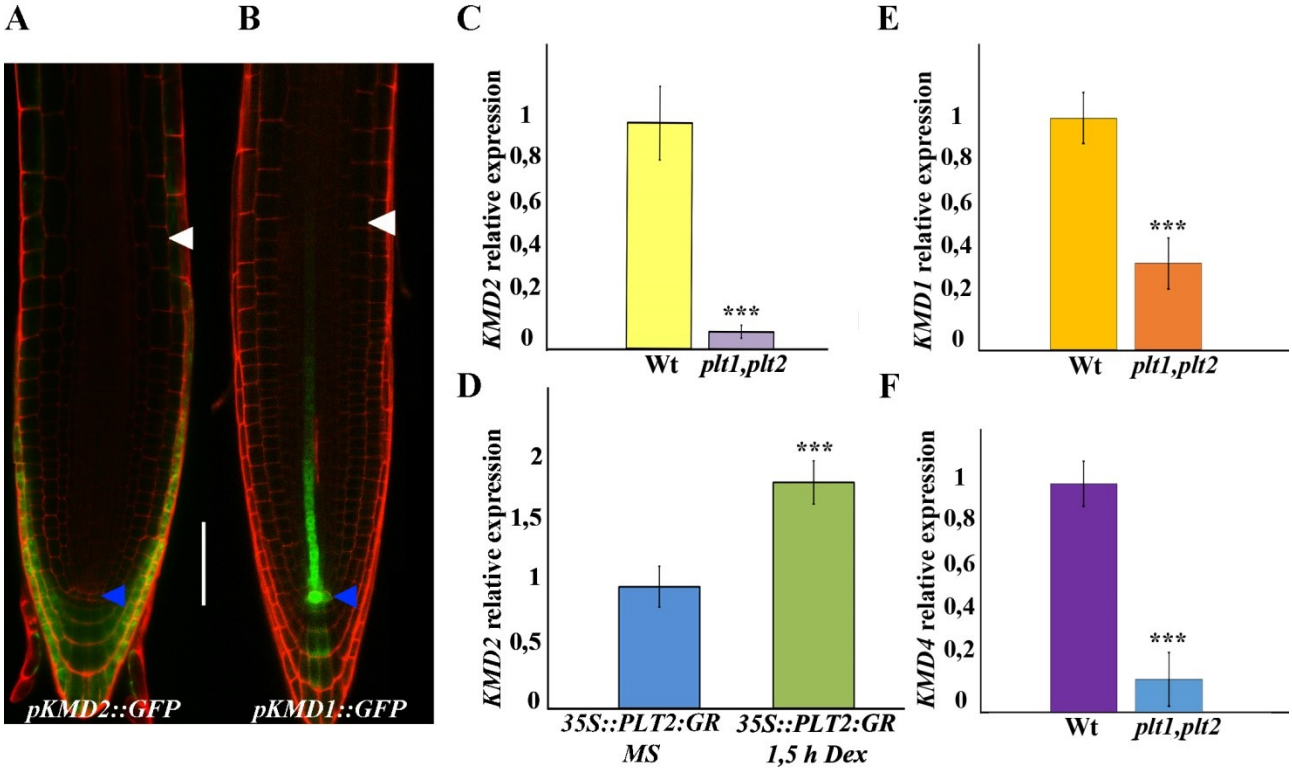

Figure 5: