

Cytokinins Determine *Arabidopsis* Root-Meristem Size by Controlling Cell Differentiation

Raffaele Dello Ioio,¹ Francisco Scaglia Linhares,¹ Emanuele Scacchi,¹ Eva Casamitjana-Martinez,² Renze Heidstra,² Paolo Costantino,¹ and Sabrina Sabatini^{1,*}

¹Dipartimento di Genetica e Biologia Molecolare
Laboratory of Functional Genomics and Proteomics
of Model Systems
Università La Sapienza
Via dei Sardi 70
00185 Rome
Italy

²Department of Molecular Genetics
Utrecht University
Padualaan 8
3584 CH Utrecht
The Netherlands

Summary

Plant postembryonic development takes place in the meristems, where stem cells self-renew and produce daughter cells that differentiate and give rise to different organ structures. For the maintenance of meristems, the rate of differentiation of daughter cells must equal the generation of new cells: How this is achieved is a central question in plant development. In the *Arabidopsis* root meristem, stem cells surround a small group of organizing cells, the quiescent center. Together they form a stem cell niche [1, 2], whose position and activity depends on the combinatorial action of two sets of genes—*PLETHORA1* (*PLT1*) and *PLETHORA2* (*PLT2*) [3, 4] and *SCARECROW* (*SCR*) and *SHORTROOT* (*SHR*) [2]—as well as on polar auxin transport. In contrast, the mechanisms controlling meristematic cell differentiation remain unclear. Here, we report that cytokinins control the rate of meristematic cell differentiation and thus determine root-meristem size via a two-component receptor histidine kinase-transcription factor signaling pathway. Analysis of the root meristems of cytokinin mutants, spatial cytokinin depletion, and exogenous cytokinin application indicates that cytokinins act in a restricted region of the root meristem, where they antagonize a non-cell-autonomous cell-division signal, and we provide evidence that this signal is auxin.

Results and Discussion

Cytokinins Regulate Root-Meristem Size

The root meristem originates from a group of stem cells, which generate cells that divide several times before entering a zone of rapid elongation without division (elongation-differentiation zone) and differentiating to maturity (Figure 1A) [5]. The rates of cell division and

elongation-differentiation are integrated so that the size of the root meristem and the rate of root growth are determined. Cell differentiation is initiated at the transition zone (TZ), encompassing the boundaries between dividing and expanding cells in the different files (Figure 1A).

Cytokinins are known as crucial signaling molecules controlling meristem activity [6–9]. To investigate a possible function of cytokinins in controlling *Arabidopsis* root-meristem size, we followed the development of the root meristem upon application of exogenous cytokinins. Root-meristem size was expressed as the number of cortex cells in a file extending from the quiescent center (QC) to the first elongated cell (Figures 1A and 1B) [10]. Untreated meristems reached their final size 5 days after germination (dpg), when a fixed number of approximately 30 cells is established in the meristem as the rate of cell division equals the rate at which cells exit the proximal meristem and enter the elongation-differentiation zone (Figure 1D).

Application of cytokinins caused a decrease in meristem size (Figures 1B and 1C) because of a progressive decrease in the number of meristematic cells (Figure 1D). Similar results were obtained with different *Arabidopsis* ecotypes (Figure 1D) and different cytokinins (Figure 1).

Cytokinins Do Not Affect Stem Cells or Root-Meristem-Division Potential

A decrease in meristem size can be caused by reduced stem cell niche activity, by loss of division potential of meristematic cells in the proximal meristem, or by a more rapid elongation-differentiation of the meristematic cells at the TZ.

We first analyzed the expression of three QC-expressed promoter traps (QC25, QC46, and QC184), expression of *SHR* and *SCR* proteins, and *PLT1* promoter activity in the root after cytokinin treatment. All were still expressed 6 dpg, when root-meristem size is already substantially reduced compared to untreated roots (Figures 1B and 1C and Supplemental Data available online). Columella stem cells did not acquire differentiation markers such as amyloplasts (Figures 1B and 1C), and they were still dividing (data not shown). Also, cortex-endodermis stem cells did not show any sign of progression in differentiation in light of the fact that they did not divide periclinally more often than the wild-type (data not shown). These data suggest that high levels of cytokinins in the root reduce meristem size without interfering with QC specification and stem cell function.

To assess whether the reduction in meristem size could be caused by loss of meristematic-cell-division potential, we visualized root-meristem cells in the G2–M phase in untreated and cytokinin-treated plants harboring the *D-Box* CYCB1::GUS construct [11]. The percentage of GUS-stained cells and the relative number of newly formed cell walls (data not shown) in the meristem was the same in cytokinin-treated and

*Correspondence: sabrina.sabatini@uniroma1.it

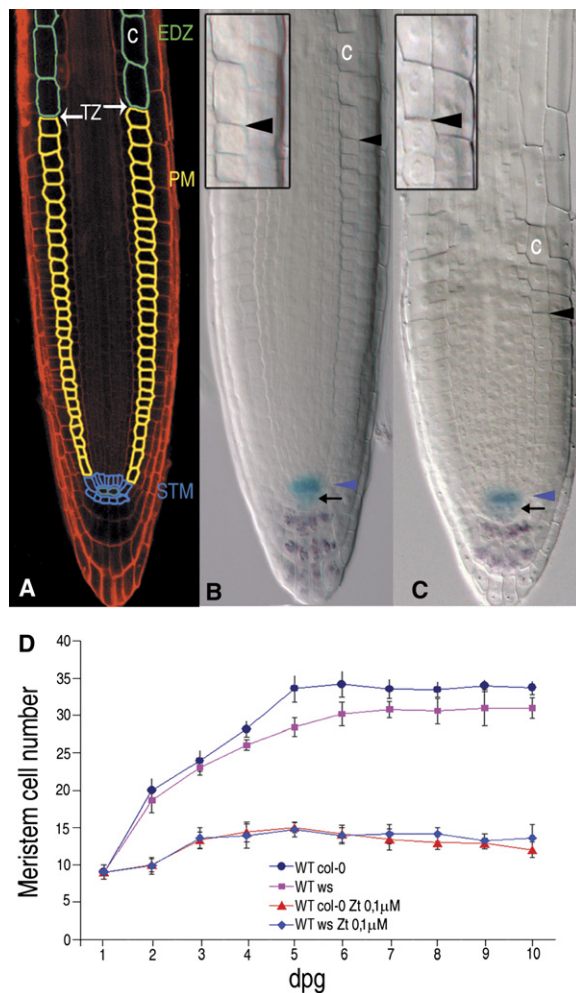


Figure 1. Exogenous Cytokinin Application Reduces Root-Meristem Size

(A) Longitudinal view of the *Arabidopsis* root meristem, where the stem cell niche is marked in blue, a cortex cell file of the proximal meristem (PM) is marked in yellow, and a cortex cell file of the elongation-differentiation zone (EDZ) is marked in green. White arrows indicate the transition zone (TZ) where cells leave the meristem and enter the EDZ. Note that the TZ is different for each cell type, giving a jagged shape to the boundary between dividing and expanding cells.

(B and C) QC25 expression (B) in wild-type root and (C) in cytokinin-treated root at 6 dpv. Double-labeling of QC (indicated by blue arrowheads) and differentiated columella cells visualized by, respectively, QC25 and amyloplast staining indicate functional QC and the presence of columella stem cells (indicated by black arrow). Black arrowheads indicate the cortex TZ; c, cortex.

(D) Root-meristem cell number of wild-type plants and wild-type plants grown on 0.1 μ M transzeatin measured over time. For monitoring root-meristem growth, cortex meristematic cells enclosed between the blue and black arrowheads (B and C) were counted. The same results were obtained with 0.1 μ M dihydrozeatin, 1 μ M kinetin, and 0.1 μ M 6-benzylaminopurine. The lowest cytokinin concentration affecting meristem size was 0.02 μ M transzeatin. The decrease in meristem size was dose dependent. For each experiment, a minimum of 90 plants were analyzed.

untreated roots, suggesting a similar overall division rate (Supplemental Data). Therefore, meristem consumption induced by high cytokinin levels cannot be explained by changes in the competence of meristematic cells to divide.

These data suggest that cytokinins control meristem size affecting the position of the TZ, by acting on the rate of meristematic cell differentiation. To verify this hypothesis, we analyzed the root meristems of cytokinin-biosynthesis and signaling mutants and of plants with local cytokinin depletions.

Cytokinin Biosynthesis Mutants Have Enlarged Root Meristems

The ATP/ADP isopentenyltransferases (*AtIPT*) catalyze the rate-limiting step of cytokinin biosynthesis in *Arabidopsis*. Each gene has a unique spatial expression pattern, with *AtIPT3*, *AtIPT5* and *AtIPT7* overlapping in the vascular tissue of the root elongation-differentiation zone [12]. The *ipt3, ipt5, ipt7* triple mutant has severely reduced levels of different types of cytokinins affecting root and shoot development [13]. *ipt3, ipt5, ipt7* root meristems showed an increased number of meristematic cells already at 2 dpv (Figure 2H) and accumulated cells also after 5 dpv, vastly exceeding the fixed number of cells of wild-type meristems (Figures 2F and 2H). The increased size of *ipt3, ipt5, ipt7* meristems (Figure 2H) correlated with an enhanced root growth rate resulting in longer roots (Figures 2G and 2I). Consistent with the notion that the root-meristem phenotype of *ipt3, ipt5, ipt7* plants is due to cytokinin shortage, cytokinin treatment restored root-meristem size to that of the wild-type (Supplemental Data). Thus, a reduction of cytokinin biosynthesis in the root correlates with an increase in meristem size and root growth rate, corroborating the hypothesis that cytokinins control the rate of root-meristem-cell differentiation.

Cytokinins Act at the TZ to Control Root-Meristem Size

To further substantiate the role of cytokinins in mediating meristematic cell-differentiation rate at the TZ, we sought to record changes in meristem size upon lowering the endogenous cytokinin level in a spatial fashion. It had been shown that constitutive expression of the cytokinin oxidase-dehydrogenase1 gene (*AtCKX1*) results in a drastic reduction of endogenous cytokinins in root and shoot meristems, and such a reduction affects root and shoot development [8].

We first expressed *AtCKX1* under the control of the *ROOT CLAVATA HOMOLOG 2* (*RCH2*) promoter, which confers a high level of expression in all tissues of the TZ (Figure 3B). The *pRCH2::CKX1* construct was introduced in plants carrying *pRCH2::GFP* for visualization of the activity of *pRCH2* (Figure 3B). *pRCH2::CKX1*, *pRCH2::GFP* plants phenocopied cytokinin-biosynthesis mutants (*ipt3, ipt5, ipt7*), with meristems larger than the wild-type already at few dpv, which continued growing after 5 dpv (Figures 3B and 3F). This suggests that low cytokinin levels at the TZ are sufficient for increasing root-meristem size.

For understanding whether low cytokinin levels must occur specifically at the TZ, *AtCKX1* was fused to the *RCH1* promoter [10], active only in the root meristem (Figure 3C). The *pRCH1::AtCKX1* construct was introduced in plants carrying *pRCH1::GFP* for visualization of the activity of *pRCH1* (Figure 3C). No changes in meristem size were detected in *pRCH1::AtCKX1* plants

Download English Version:

<https://daneshyari.com/en/article/2045260>

Download Persian Version:

<https://daneshyari.com/article/2045260>

[Daneshyari.com](https://daneshyari.com)