Nitrate modulates stem cell dynamics in Arabidopsis shoot meristems through cytokinins

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The shoot apical meristem (SAM) is responsible for the generation of all the aerial parts of plants. Given its critical role, dynamical changes in SAM activity should play a central role in the adaptation of plant architecture to the environment. Using quantitative microscopy, grafting experiments, and genetic perturbations, we connect the plant environment to the SAM by describing the molecular mechanism by which cytokinins signal the level of nutrient availability to the SAM. We show that a systemic signal of cytokinin precursor mediates the adaptation of SAM size and organogenesis rate to the availability of mineral nutrients by modulating the expression of WUSCHEL, a key regulator of stem cell homeostasis. In time-lapse experiments, we further show that this mechanism allows meristems to adapt to rapid changes in nitrate concentration, and thereby modulate their rate of organ production to the availability of mineral nutrients within a few days. Our work sheds light on the role of the stem cell regulatory network by showing that it not only maintains meristem homeostasis but also allows plants to adapt to rapid changes in the environment.

Abbradopsis | shoot apical meristem | plant nutrition | plant development | cytokinin hormones

Plants have evolved specific mechanisms to adapt their growth and physiology to the availability of mineral nutrients in their environment (1). Various hormones such as auxin, abscisic acid, gibberellin, and cytokinin have been shown to act in this process either locally or systemically (1). Cytokinins in particular play an essential role in plant response to nitrate, where they act as second messengers (2). For example, cytokinins promote lateral root development in areas rich in NO\(_3\) if the overall NO\(_3\) availability for the plant is low (3). In the shoot, cytokinins have been shown to modulate key traits such as leaf size (4, 5) and branch number (6) in response to nitrate. Cytokinins have also been shown to be critical for the maintenance of stem cell homeostasis in the shoot apical meristem (SAM). By modulating the expression of WUSCHEL (WUS), encoding for a homeodomain transcription factor expressed in the center of the meristem, cytokinins promote stem cell proliferation, thus controlling the size of the meristem and the rate of shoot organogenesis (7–10). Using grafting experiments, a recent study showed that a systemic signal of a cytokinin precursor \(\text{trans-zeatin riboside} (\text{IZR})\), traveling from root to shoot through xylem, could influence the size of the vegetative meristem (11). However, it remains unclear whether cytokinin signaling can allow the SAM to respond to changes in nutrient levels in the environment. Here, we examined how a core stem cell regulator in the SAM dynamically responds to changes in mineral nutrient levels in the soil and whether systemic cytokinin signals can account for the dynamic adaptation of meristem function to nutrient levels. We used the inflorescence meristem of Arabidopsis as a model, as this structure produces all the flowers of the plant, and is therefore a key target for crop improvement.

\(\text{Fig. S2 and SI Material and Methods}\). The intensity of the signal and the size of the domain of

Significance

Plants generate organs throughout their life as a consequence of the maintenance of postembryonic stem cell niches in meristems. The molecular mechanisms controlling stem cell homeostasis and organ emergence in shoot meristems have been well described, but the manner in which environmental signals influence them to generate plasticity is largely unknown. Using the shoot apical meristem of Arabidopsis as a model system, we show that plants can adapt their organogenesis rate to changes in the availability of nitrate in the soil within a few days, thanks to long-range signaling by cytokinin hormone precursors that travel through the plant, are converted to active hormones that travel to shoot meristems, and modulate the expression of WUSCHEL, a key regulator of stem cell homeostasis.

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Plant nutritional status influences stem cell homeostasis in the SAM. 

Cytokinins Are Involved in the Response of the Meristem to Mineral Nutrients. First, we looked at the phenotype of mutants altered in the successive steps of production of cytokinins via ISOPENTENYL TRANSFERASE (IPT) and LONELYGUY (LOG) enzymes (19): ipt3-1 ipt5-2 ipt7-1 (referred to here as ipt3.5.7) for the first step of biosynthesis and log4-3 log7-1 (log4.7) and log1-2 log3-2 log4-3 log7-1 (log1.3.4.7) for the second step of biosynthesis; for mutants for the conversion of cytokinins via CYTOCHROME P450 MONOOXYGENASE 735A (CYP735A) enzymes (20): cyp735a1-1 cyp735a2-1 (cyp735a1.2); and for the degradation of cytokinins via CYTOKININ OXIDASE (CKX) enzymes (13): cka3-1 cka5-2 (cka3.5) in plants grown on soil supplied with fertilizer. Mutant shoots grew almost normally in this condition, and only the ipt3.5.7 and cyp735a1.2 mutants showed a consistent decrease in rosette weight (Fig. 3A and SI Appendix, Fig. S6A). However, meristem size...
Cytokinin precursors act as long-range signals in the control of SAM homeostasis. (A) Representative meristems of WT and cytokinin-associated mutant plants self-grafted or grafted with a WT rootstock and grown on soil supplied with fertilizer. (Scale bar, 50 μm.) (B) Genotype of the rootstock (WT or ipt3.5.7 mutant). (C) Genotype of the scion (WT or ipt3.5.7 mutant). Data were compared using Student’s t-tests. Error bars correspond to the mean ± SD.

Fig. 3. Cytokinins allow the adaptation of meristem function to plant nutritional status. (A) Morphology of the rosette (Top) and of the SAM (Bottom) of representative WT and CK-associated mutant plants. (Scale bar, Top, 1 cm; Bottom, 50 μm.) (B and C) Meristem size (B) and plastochron ratio (C) of WT and CK-associated mutant plants grown on soil either without fertilizer (green, g) or with fertilizer (red, r); (Col-0: n = 22 (g) and 25 (r); ckx3.5: n = 23 (g) and 27 (r); log4.7: n = 19 (g) and 24 (r); log1.3.4.7: n = 14 (g) and 25 (r); ipt3.5.7: n = 36 (g) and 20 (r); cyp735a1.2: n = 26 (g) and 20 (r), pool of two independent experiments). Data were compared using Student’s t test. Error bars correspond to the mean ± SD.

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Cytokinin Precursors Act as Long-Range Signals in the Control of Meristem Function. We further studied the spatial origin of the signal triggering the response of the meristem to mineral nutrition. Grafting experiments have previously shown that cytokinins can act either as local or as systemic signals in the control of root and organogenesis rate were strongly affected by the mutations (Fig. 3 and SI Appendix, Fig. S6 B–D). The ipt3.5.7, log4.7, log1.3.4.7, and cyp735a1.2 mutants, altered in various steps of trans-zeatin (tZ) production, showed smaller inflorescence meristems that produced fewer organs. In contrast, the ckx3.5 mutant, which displays higher levels of cytokinins, showed larger inflorescence meristems that produced more organs, as previously described (13). We also quantified the size of the domain of expression of WUS by in situ hybridization (SI Appendix, Fig. S7). We observed changes in the size of the WUS expression domain in all genotypes that correlated with the changes in meristem size we quantified, thus supporting the idea that cytokinin metabolism controls meristem function through the stem cell regulatory network.

We next looked at the effect of mineral nutrition on the phenotype of the mutants by comparing plants grown on soil with or without fertilizer. Of all mutants, only ipt3.5.7 mutants did not respond to fertilizer with an increase in meristem size and organogenesis rate, as observed in WT. Instead, it displayed a surprising statistically significant decrease in meristem size (Fig. 3). Although all the enzymes involved in cytokinin metabolism we studied can modulate meristem homeostasis, IPT enzymes, which catalyze the first step of cytokinin production, appear to be the only ones necessary for the response of the meristem to changes in nutrient availability in the soil. This result is supported by the fact that nitrate, the main mineral nutrient, can directly modulate the expression of IPT3 and, to a lesser extent, of IPT5 in seedlings (21). To further support that the response of meristems to mineral nutrients relies on cytokinin precursors produced by IPT enzymes, we used mass spectrometry to compare CK levels in inflorescences of plants grown with or without fertilizer. Despite technical variability in the mass spectrometry measurements between replicates (SI Appendix, SI Material and Methods), we observed a statistically significant increase in the levels of tZR precursors, the dephosphorylated products of IPT enzymes, in the two experimental replicates, further supporting the importance of these enzymes in the response of the meristem to nutrients (SI Appendix, Fig. S8 and Tables S2 and S3). We did not find significant changes in the levels of the active cytokinin tZ in the whole inflorescence, but found a significant increase in the levels of the degradation products of tZ: tZROG (trans-zeatin-O-glucoside riboside) and tZG (trans-zeatin-7-glucoside) (SI Appendix, Tables S2 and S3). This lack of changes in tZ levels could be a result of tZ being a transient molecule mainly synthesized locally in the meristem, notably through the action of LOG4 and LOG7 enzymes (7–9).
and shoot development (11, 20, 22). We performed grafting experiments on our set of mutants to study whether a systemic signal is involved in the control of meristem function. Adding a WT root was able to rescue the phenotype in the meristem of ipt3.5.7 and cyp735a1.2 mutants, but not of the cka3.5 and the two log mutants (Fig. 4 A and B and SI Appendix, Fig. S9). Grafting a mutant root onto a WT scion led to a minor but statistically significant decrease in meristem size for log1.3.4.7, ipt3.5.7, and cyp735a1.2 mutant roots (SI Appendix, Fig. S10). These experiments show that expressing IPT and CYP735A in either part of the plant is sufficient but not completely necessary for proper meristem function, and that LOG and CKX must act largely in the shoot to regulate meristem function. Our results are in agreement with the recent work by Osugi et al. (11), who showed that the phenotype in the vegetative meristem of the ipt triple mutant, but not the log sextuple mutant, could be complemented by grafting a WT root. These results also make sense in light of the expression patterns of the different genes, as LOG and CKX are expressed in meristems (7, 9, 13), whereas IPTs and CYP735a1-2 are expressed predominantly in the vascular tissues of the root and, to a lesser extent, of the shoot (16, 23); only IPT7 expression has been shown to be induced by SHOOT MERISTEMLESS in the vegetative meristem (24). We next looked at the response to mineral nutrition of grafted combinations of WT and ipt3.5.7 mutant plants. Contrary to self-grafted mutants, ipt3.5.7 mutant plants grafted with WT root or WT shoot and growing with fertilizer showed larger meristems and reduced plastochron ratios than the same plants growing without fertilizer (Fig. 4C). IPT activity exclusively in the root or solely in the shoot is thus sufficient, but not necessary, for the meristem to respond to changes in the nutritional environment.

To analyze the dynamics between production of cytokinin precursors and modulation of meristem function, we applied tZR and tZ to meristems expressing pTCSn::GFP and pWUS::GFP cut from the plant and grown in vitro. In the absence of added cytokinin, both pTCSn::GFP and pWUS::GFP signal strongly decreased over a 48-h time course, suggesting that extrinsic cytokinin is needed to sustain meristem homeostasis (Fig. 5). In contrast, adding 50 μM either of tZR or tZ to the medium up-regulated pTCSn::GFP expression ectopically and allowed the maintenance of the pWUS::GFP signal in the center of the meristem. Inducing IPT3 in cut meristems in the absence of extrinsic cytokinin also maintained, at least partially, WUS expression (SI Appendix, Fig. S11). Together with the results from the grafting experiments, these data support a model in which stem cell homeostasis is controlled by a systemic signal of cytokinin precursors (tZR) produced by IPT enzymes outside the meristem. As tZ alone cannot trigger cytokinin response (11), this systemic signal must be locally converted to active cytokinins by LOG enzymes in the shoot meristem.

**Cytokinins Allow a Rapid Response of Meristems to Changes in Nitrate Availability.** Nitrogen is a major nutrient that can modulate various aspects of plant development (25), and the availability of which in the soil can vary rapidly during the lifecycle of a plant (26). Adding nitrate (NO₃⁻) to nitrogen-deficient plants leads to a rapid and transient increase in cytokinin levels (11, 21, 27, 28). Given the rapid response of meristems to changes in cytokinin levels we observed in vitro, we looked to see whether the meristem could dynamically respond to changes in nitrate concentration in vivo. We grew plants on sand, watering them with a nutritive solution containing a low concentration of nitrate (1.8 mM NO₃⁻ as the only source of nitrogen) to induce a deficiency (6). Once the plants bolted, we watered them with a nutritive solution containing different concentrations of NO₃⁻ (0, 1.8, or 9 mM) and quantitatively analyzed the dynamics of response to this treatment. In the SAM, the treatment led to the dose-dependent induction of pTCSn::GFP and of pWUS::GFP expression within only 1 d, and to an increase of meristem size and of the organogenesis rate within 2–3 d (Fig. 6 A–C and SI Appendix, Fig. S12). Similar to what was observed when plants

Fig. 5. Cytokinin precursors are sufficient to maintain meristem homeostasis in vitro. Effect of tZR and tZ application on the expression of pTCSn::GFP(A) and pWUS::GFP (B) in cut meristems grown in vitro (pools of two independent experiments, pTCSn::GFP: n = 11 for each condition, pWUS::GFP: n = 12 for each condition). (Top) Representative plants. (Scale bars, 50 μm.) Red arrows point to the center of the inflorescence meristem. (Bottom) Total fluorescence signal in the inflorescence meristem at 24 h and 48 h divided by the signal at 0 h. Data were compared using Student’s t test. Error bars correspond to the mean ± SD.
were grown on soils of different nutritive qualities, the nitrate treatment affected the size of CLV3 expression domain more than its levels (SI Appendix, Fig. S13). We then checked whether this response relied on changes in cytokinin levels in the plant. In the root, we confirmed that the addition of nitrate to deficient plants led to a rapid, dose-dependent, and transient induction of nitrate signaling (inferred from the level of expression of the nitrate responsive gene NITRATE REDUCTASE 1) and of IPT5 (SI Appendix, Fig. S14A). By measuring the levels of cytokinin species 2 d after the treatment by mass spectrometry, we also confirmed that the treatment led to a significant increase in the concentration of cytokinin precursors (tZR and/or trans-zeatin ribose phosphate, depending on the tissues) and products of degradation (tZ7G, tZ9G, and/or tZROG, depending on the tissues; SI Appendix, Fig. S14B and Table S4). Finally, we analyzed the meristem response in our set of mutants 3 d after a nitrate treatment (9 mM NO₃). Similar to what we observed on plants grown in soil, the response of ipt3.5.7 mutant plants to nitrate in the meristem was strongly reduced, although statistically significant in one of the two experimental repeats, whereas response in the other mutant backgrounds was as in WT (Fig. 6D and SI Appendix, Fig. S15).

Discussion
Taken together, our results show that the meristem can adapt the rate of shoot organogenesis to the availability of nitrate in the soil. Mechanistically, this phenomenon correlates with the ability of WUS and cytokinin signaling output to quantitatively respond
to variations in nitrate levels. As the main inflorescence of WT plants growing on soil only produced an average of 3.1 ± 0.4 flowers per day (n = 16), the response of the SAM to changes in nitrate levels, which leads to significant changes in the rate of organ production in only 2 d, should be seen as very rapid in comparison with the pace of morphogenesis in this tissue. The timing of the response to nutrients is very similar to the response to transient perturbations of the core network (29), in both cases causing expression domain changes in a day followed by changes in growth and size. Our findings thus expand the understanding of the function of the stem cell regulatory network in the SAM. They show that this network not only acts to maintain the integrity of the SAM during organogenesis but also allows a very rapid adaptation of SAM function to a nutritional cue.

A recent study showed that vegetative meristems of seedlings germinating in the dark displayed reduced WUS expression, which was controlled by the activity of CKX enzymes (12). Given the pleiotropic effects of cytokinins (2), we can hypothesize that different environmental signals could be integrated through cytokinin signaling and lead to different developmental responses in the meristem depending on whether they modulate, locally or globally, different aspects of cytokinin signaling.

Although it was developed in Arabidopsis, this model can in the future be applied to crops, where cytokinin metabolism and action on stem cell regulation should be conserved. In rice, where LOG mutants were first characterized (15), quantitative trait loci for increased grain productivity have been mapped to genes involved in the regulation of cytokinins, including notably a CKX enzyme-encoding gene (30, 31). In maize, weak mutants of FASCIATED-EAR3, a receptor able to bind a CLV3 homolog and involved in the control of WUS expression, also shows increased seed yield (32). Our work, which provides an integrative model based on nutrient availability, cytokinin metabolism, and its effect on stem cell regulation and meristem function ([SI Appendix, Fig. S16]), allows a better characterization of the influence of mineral nutrients on plant architecture and could be used to better understand plant response to environmental inputs and to develop cultivars with increased yield.

Methods

Detailed information on plant material, growth conditions, construction of inducible lines, image acquisition and analysis, grafting, mass spectrometry, qPCR, and in situ hybridization is provided in SI Appendix, Materials and Methods.

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