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Control of shoot and root meristem function by cytokinin

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Plant hormones regulate a variety of processes fundamental for growth and development. Recent studies have clearly shown that establishing adequate spatial and temporal distribution of hormones is central in the control of development. The activity of cytokinins (CKs) is essential to maintain undifferentiated cells in shoot apical meristem (SAM) and to promote cell differentiation in the root meristem (RAM). Detailed mechanisms how the gradient of CK activities is established in the meristem has begun to be elucidated.

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Introduction

Cytokinins (CKs), naturally occurring plant hormones that promote cell division, are essential for normal plant growth and development [1,2]. Since Skoog and Miller demonstrated the ability of CKs to induce shoot regeneration from tissue cultures in 1957, CKs have been implicated in the control of shoot meristem activity [3], but direct evidence for this implication had been provided only recently [4,5]. More recently, studies have begun to indicate the importance of CK localization in the control of shoot meristem function. This review describes how spatial and temporal distribution of CK activity in the shoot meristem is established through a complex regulation of multiple steps in CK metabolism and signaling. A possible involvement of CKs in the control of meristem identity is also discussed.

Maintenance of shoot meristems by CK

The growth of plants depends on the continuous function of meristems where a balance between cell proliferation and differentiation is properly coordinated. The first evidence of *in planta* CK function in cell proliferation in the SAM was obtained from the analysis of CK-deficient transgenic plants overexpressing CK oxidase/

dehydrogenase (CKX) genes, which degrade active CKs [4,5]. Subsequently, a severe reduction in the size of shoot meristems in a mutant defective in all of its CK receptors in *Arabidopsis* has provided compelling evidence that CK is required for meristem activity [6,7]. Triple, quadruple and quintuple mutants of *Arabidopsis* adenosine phosphate-isopentenyltransferase (IPT) genes, which catalyze the first and rate-limiting step of CK biosynthesis [8,9], caused a significant decline of CK levels and resulted in the reduction of SAM size, further supporting the positive role of CKs in the control of shoot meristem activity [10]. However, in all these studies, overall CK levels or signaling was depleted and consequently plants showed a series of CK-related abnormalities besides the defects in the SAM. Thus, whether CKs are specifically involved in the control of SAM maintenance still remained to be determined.

Specific role of CKs in the maintenance of SAM

Like the other plant hormones, CK activity *in planta* is controlled by a balance between several factors, such as synthesis, degradation, inactivation, reactivation, and so on [1,11]. Moreover, CKs are supposed to be involved in long-distance translocation through the selective transport system via xylem and phloem, making the mechanism of regulation of CK activity more elaborate. Recent studies have begun to uncover mechanistic links between transcription networks controlling the SAM activity and hormone biosynthesis and signaling [12,13,14,15–19]. KNOTTED1-like homeobox (KNOX) proteins are central players in the control of SAM [20,21]. They ensure the maintenance of SAM by repressing differentiation of cells in the SAM. Intriguingly, KNOX proteins function through controlling the balance of plant hormones, particularly CK and GA levels to establish a high CK to GA ratio in the SAM [17,18]. To achieve this condition, KNOX directly suppresses GA20-ox, a GA biosynthesis gene [12,13] and activates IPT genes [14,15,16]. Because KNOX expression is restricted in shoot meristem cells [20–22], this regulation is effective in ensuring high CK/low GA condition in a spatially controlled manner.

IPTs are encoded by a small gene family with at least seven and eight members in *Arabidopsis* and rice, respectively [8–10,16]. Each member of the family shows distinct spatial expression and responsiveness to hormones [8,9,16]. In both species, a subset of the IPT genes is recruited as targets of KNOX proteins. In rice, transcription of *OsIPT2* and *OsIPT3* is induced by OSH1, a KNOX protein [22]. *OsIPT2* and *OsIPT3* are also unique in that they are not negatively regulated by CK while the rest of

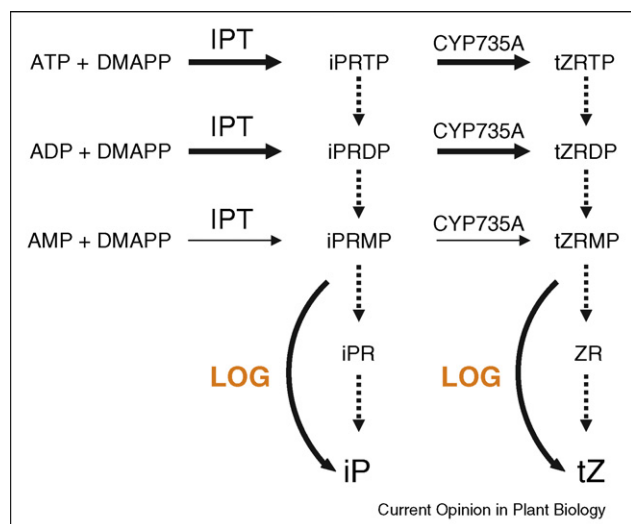
the rice IPT genes are [16]. An attractive explanation for this observation would be that the negative regulation by CK is cancelled for these two genes to assure a rigorous regulation by KNOX. In *Arabidopsis*, *AtIPT7* is the only IPT gene controlled by KNOX [14^{••},15], however, *ipt7* mutants do not show a visible phenotype [10]. On the other hand, the meristem size is reduced significantly when other *ipt* mutations are combined with *ipt7* [10], indicating that other IPT genes are also involved in the control of meristem function in spite that they are not induced by KNOX [14^{••},15]. This implies that both KNOX-dependent, cell type specific control and KNOX-independent CK synthesis contribute to maintaining SAM.

Control of SAM activity by CK biosynthesis

Recently, isolation of a new CK-deficient mutant of rice, *lonely guy* (*log*), has revealed a new aspect of the mechanisms that link CKs and SAM function [23^{••}]. *LOG* encodes a novel enzyme with phosphoribohydrolase activity, which directly converts inactive CK nucleotides, such as iPRMP and tZRMP, to the bioactive free-base form with the release of a ribose 5'-monophosphate (Figure 1, [23^{••}]). *LOG* shows specific expression at the top of shoot meristems where stem cells reside [23^{••}]. Thus, active CKs are diminished locally at the top of the shoot meristem in *log* mutant, leading to perturbed

meristem functions, such as a remarkable reduction of SAM size. This reduction of SAM size is caused by a decrease of cell number, but not cell size (Sasaki E. and JK, unpublished results), demonstrating that active CKs are indeed specifically required in the proliferation of undifferentiated meristematic cells in the SAM. Moreover, defects are observed in the inflorescence meristem and in the floral meristem. *log* generates an extremely small panicle containing flowers with a reduced number of floral organs, which is caused by premature termination of the floral meristem activity [23^{••}]. Defects in *log* mutants imply that the spatially restricted conversion from inactive to active CKs by LOG provides another level of assurance to the strict control of CK function in shoot meristems. Because CKs are supposedly translocated and preserved as inactive nucleosides forms [11[•]], the control of CK levels at the final activating step may provide a powerful and secure system to generate a gradient of CK activity that works as a local paracrine signal for the shoot meristem function. In addition, it would provide a mechanism for preventing cytokinin action in cells and tissues where it is not needed. In this context, it will be interesting to determine how the specific expression of *LOG* is controlled and whether there is a link between *LOG* and the known transcription networks.

Figure 1



Schematic representation of cytokinin biosynthesis and activating pathway. An isoprenoid moiety of dimethylallyl diphosphate (DMAPP) is added to either ATP, ADP or AMP by adenosine phosphate-isopentenyltransferases (IPTs), giving rise to N^6 -(D^2 -isopentenyl)adenine (iP) riboside 5'-tri- [di or mono-] phosphate (iPRT(D,M)P). Plant IPTs prefer ATP and ADP as acceptors of DMAPP. The CK nucleotides (iPRT(D,M)P) are converted into the corresponding trans-zeatin (tZ)-nucleotides (tZRT(D,M)P) by CYP35A. De-phosphoribosylation of CK nucleotides catalyzed by LOG generates CK nucleobases, active forms of CKs. Solid arrows indicate reactions whose genes for the enzyme are identified, and dotted arrows indicate reactions whose responsible genes are unknown.

CK controls the rate of meristematic cell differentiation in the root meristem

In contrast to the positive role of CKs on proliferation of meristematic cells in the SAM, an opposite effect of CK on root growth had been observed in transgenic *Arabidopsis* and tobacco that have reduced CK level by the constitutive expression of *CKX* genes [4,5]. Recently, the detailed basis of this observation has been analyzed [24^{••}]. An increase of root meristem size was observed in the *ipt3, ipt5 ipt7* triple mutant and the *ahk3* receptor mutant that have a reduced CK biosynthesis or signaling [24^{••}]. Based on a detailed characterization of the enlarged root meristems, Dello Ioio *et al.* showed that the differentiation rate of meristematic cells are decreased in mutants and this leads to the accumulation of meristematic cells in the root meristem [24^{••}]. Thus, in roots, CKs control the rate of meristematic cell differentiation. Dello Ioio *et al.* further indicated that this function is mediated in a spatial manner by CKs specifically localize in the vascular tissue at the transition zone, a border between the root meristem and elongation-differentiation zone [24^{••}].

Control of CK signaling for shoot meristem maintenance

Biosynthesis is not the only pathway involved in the control of CK function. CK signaling is also a target of the regulatory network controlling meristem activity. *ARR5*, encoding a typeA response regulator, a CK induced negative regulator of CK signaling [25], has been

shown to be directly suppressed by WUSCHEL (WUS) in the SAM [19]. WUS, encoding a homeodomain protein, positively regulates proliferation of stem cells in *Arabidopsis* SAM [26]. Considering a highly cell-specific localization of WUS and the characteristic of ARR5 as a negative regulator of CK signaling, suppression of ARR5 by WUS is probably effective to give further spatial restriction of CK signaling in the SAM. The relevance of this regulation was confirmed by an experiment showing that constitutive expression of a dominant negative version of ARR5 mimicked *wus* phenotype [19]. ABPH1 gene of maize, encoding a typeA response regulator, shows a specialized localization of its mRNA in the SAM and the SAM is enlarged in the *abph1* mutants [27]. This also demonstrates how crucial cell type specific control of CK signaling is for SAM function.

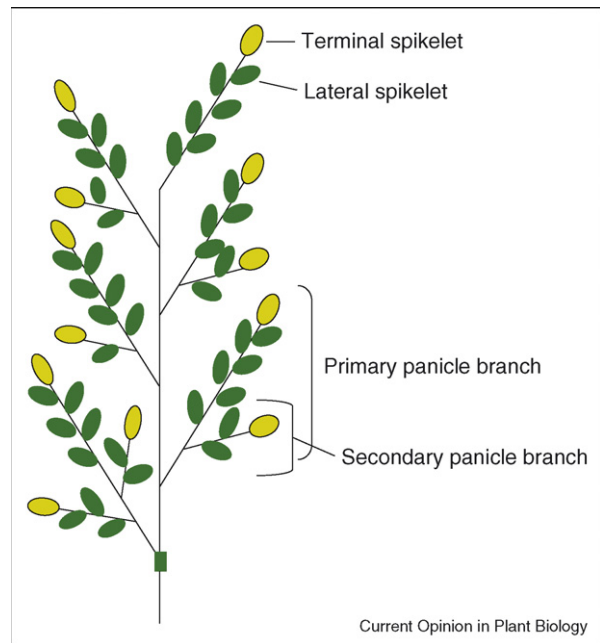
Possible involvement of CK in the control of shoot meristem identity

The pattern of shoot branching is a major factor determining plant architecture [28]. Although two steps, axillary bud generation and its outgrowth, are generally described as major determinants of the branching pattern, control of meristem identity is also a key in defining the branching pattern. A rice panicle is composed of several primary panicle branches, each of which has a few secondary panicle branches at the base, several lateral spikelets at the distal part and a terminal spikelet at the top (Figure 2). The spikelet is the ultimate unit of the grass inflorescence and in rice it comprises a single flower. Because the branch meristem is ultimately converted into a terminal spikelet meristem after it generates an appropriate number of lateral spikelets, the timing of this conversion primarily determines the number of spikelets generated on a branch.

In the *log* mutants, despite the severe reduction of branch size, each branch has a terminal spikelet at the top, indicating that the conversion of the meristem identity from a branch into a spikelet meristem occurs, but precociously [23•]. This raises a possibility that CKs might participate in the control of meristem identity, in particular, to maintain the identity as the branch meristem.

Grain number per panicle is an important agronomic trait in rice. *Grain number1a* (*Gn1a*), a QTL locus that controls grain number in rice was isolated and shown to encode OsCKX2, a CK degradation enzyme [29•]. The negative correlation between *OsCKX2* expression level and grain number indicates a positive role of CKs on grain number [29•]. The increase in grain number in high grain number cultivars was interpreted as a consequence of an increased rate of spikelet primordial formation [29•], however, this may not be the only explanation. In cultivars with high grain number, the number of lateral spikelets on each primary branch, as well as the number of secondary

Figure 2



A schematic drawing of a rice panicle. A rice panicle has several primary panicle branches. A few secondary panicle branches and several lateral spikelets are produced on each primary panicle branch. Eventually, the SAM of each panicle branch is transformed into a terminal spikelet. The timing when the branch meristem is converted to the terminal spikelet meristem is a major determinant of the number of spikelets produced in a panicle.

branches is significantly increased. Thus, an alternative explanation could be that the branch meristem identity is prolonged in high grain number cultivars, resulting in the production of more spikelets and secondary branches, and this trait is controlled by CK. This scenario is consistent with the situation in *log*, in which lower level of active CKs causes a premature conversion of the branch meristem to the terminal spikelet meristem. A small panicle has been reported in transgenic rice plants that constitutively express *OsARR6*, a typeA response regulator, a negative regulator of CK signaling [30]. Although details of the panicle phenotype are yet to be described, the appearance of the panicle in the transgenic plants suggests that a precocious transition of meristem identity has occurred [30]. Taking these observations together, there is a possibility that CKs are involved in the control of not only the maintenance of undifferentiated cells but also the identity of the shoot meristem. Currently, it is not known whether this can be extended to other types of meristems, such as the transition from a vegetative SAM to the inflorescence meristem, and to other plant species. CK action might be required in reorganization of cell files and cell division patterns during the change of meristem shape, which often accompanies the conversion of meristem identity.

Conclusions and future directions

Recent studies have clearly shown that establishment of a proper spatial and temporal distribution of CK activity is crucial for its role to maintain undifferentiated meristematic cells in the SAM. In this review, we have seen that multiple steps in CK metabolism and signaling are involved in the regulation of CK activity in the SAM. However, despite the remarkable progress made in our understanding of CK biosynthesis and function, we know little about the actual *in planta* distribution of active CKs. Considering the emerging complexity of the control of CK distribution, predicting *in vivo* localization of CK activity from the analysis of a single step or a gene may be almost impossible. Development of new tools to directly visualize CK activity at a single cell resolution would be essential for the comprehensive understanding of the CK role to meristem function. Moreover, further understanding of genes involved in the CK metabolic pathways would be a prerequisite for a better understanding of CK function during development.

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