regulation of proteasome activity by auxin [9] (Figure 1). However, the mechanism of auxin-induced redistribution of PTRE1 remains elusive. Recent exciting studies showed that the AUXIN-BINDING PROTEIN 1 (ABP1)-TMK auxin-sensing system activates the ROP signaling pathway, which affects the cytoskeleton and endocytosis without any changes to gene transcription [11]. Moreover, ABP1 mainly localizes to plasma membrane and ER. Therefore, it will be interesting to investigate whether ABP1 mediates the auxin-regulated redistribution of PTRE1, although the role of ABP1 as an auxin receptor remains controversial [12].

Concluding Remarks and Outstanding Questions

In summary, these results represent a significant step toward understanding the regulation of Aux/IAA degradation in auxin signaling, and uncover a new regulatory mechanism that contributes to auxin signaling in plants. The rapid degradation of Aux/IAAs by TIR1 in response to auxin could be balanced through the PTRE1-dependent feedback regulation of proteasome activity, preventing the exaggerated degradation of Aux/IAAs and overamplified auxin signaling (Figure 1). Nevertheless, some questions remain. The wide expression pattern of *PTRE1* indicates that this proteasome inhibitor protein is not only involved in auxin signaling, but is also associated with several other hormone signaling pathways. Although the authors showed that the *ptre1* mutant is hypersensitive to brassinolide (BL) and ABA treatment, they did not detect the accumulation of RGA1, which has a key role in GA signaling in *ptre1*, suggesting that PTRE1 selectively regulates proteasome activity. In addition, whether the transmembrane domain is required for the functionality of PTRE1 is still debated. Thus, detailed studies of the effect of deleting the transmembrane region of PTRE1 on IAA degradation and 26S proteasome activity will help us to understand how auxin regulates the redistribution of PTRE1. Another significant challenge will be to understand how PTRE1 controls proteasome activity.

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Spotlight

The Yin–Yang of Cytokinin Homeostasis and Drought Acclimation/Adaptation

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Increasing evidence has shown that cytokinins (CKs) regulate plant drought acclimation/adaptation through a multistep phosphorelay pathway. Recent progress has allowed us to suggest a yin-and-yang-type relationship between CK homeostasis and acclimation/adaptation responses that modulates plant fitness and yields stability under drought through a complex network involving cross-talk with abscisic acid (ABA).

CK Metabolism and Signaling in Plants

CKs play an important role in the regulation of various biological processes in plants, including growth and development as well as acclimation/adaptation to environmental stresses [1]. In the past 20 years, identification of key genes involved in CK metabolism has allowed us to form a detailed picture of CK metabolism in plants. CK homeostasis in plant cells is tightly regulated by the concerted actions of both isopentenyltransferase (IPT) and CK oxidase/dehydrogenase (CKX) enzymes, depending on the plant developmental stage and growing conditions. IPTs are key CK biosynthetic enzymes catalyzing the first and rate-limiting step of isoprenoid CK biosynthesis, whereas CKXs control CK homeostasis via CK degradation. A total of nine *IPT* (*IPT1–9*) and seven *CKX* (*CKX1–7*)

genes were identified in the model *Arabidopsis thaliana* [1,2].

In *Arabidopsis*, CK signaling involves a multistep His–Asp phosphorelay comprising three CK receptor histidine kinases (AHK2, AHK3, and AHK4), five authentic histidine phosphotransfer proteins (AHP1–5), and 21 response regulators (ARRs) that are further classified into ten type A and 11 type B [2]. On perceiving CKs, the AHKs are autophosphorylated and the phosphoryl group is then transferred to the acceptor side of the AHKs. AHPs receive the phosphoryl groups from the AHKs and transfer them to nuclear type B ARRs, which regulate the expression of downstream target genes including type A *ARR*s. The AHPs also phosphorylate type A ARRs, which can control the activities of type B ARRs through competition for phosphorylation and feedback inhibition of CK signaling. Additionally, several members of the AP2/ERF-type transcription factor (TF) and F-box families, called CK response factors (CRFs) and Kiss Me Deadly (KMD) proteins, were identified in *Arabidopsis* as components of CK signaling. Details of CK metabolism and signaling can be found in [1,2].

Plant Drought Adaptation Through Controlling CK Homeostasis and CK Signaling

To cope with unfavorable water-deficit conditions, plants use sophisticated and complex mechanisms to adjust endogenous CK levels to appropriately control the CK signal transduction pathway and its downstream genes. In the past several years, intensive research has been conducted to find an answer to how plants control CK homeostasis and the CK signaling network for acclimation and adaptation on perceiving drought. A growing body of evidence has indicated the multifaceted nature of CKs; that is, CKs can have both negative and positive effects on drought tolerance [1,3]. Likewise, CK levels may decrease or increase depending on stress duration or intensity [3]. The positive effect of CKs on drought tolerance was elegantly evidenced by a carefully designed enhancement of endogenous CK levels in transgenic plants that led to improved drought tolerance through delaying senescence [4].

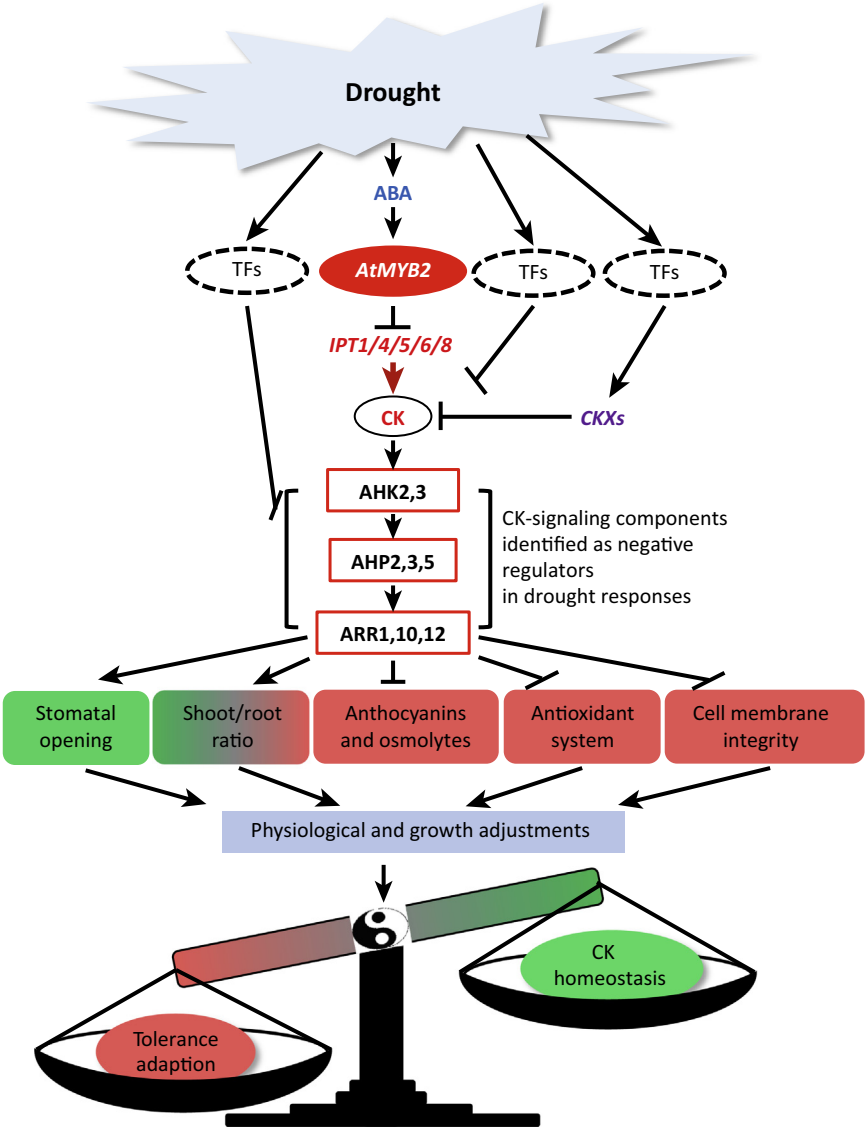

Figure 1. Model for the Roles of Cytokinins (CKs) and CK Signaling in Plant Acclimation/Adaptation to Drought Stress: A Yin-and-Yang Relationship between CK Homeostasis and Plant Acclimation/Adaptation to Drought. On exposure to drought, abscisic acid (ABA) accumulates in plants, which induces expression of *AtMYB2* [9]. Upregulation of *AtMYB2* results in downregulation of several *IPT* genes [10] and, as a consequence, endogenous CK levels. The reduction in CK content attenuates the regulatory effects of CK signaling on certain physiological and biochemical processes, which results in adaptive physiological and growth adjustments (e.g., enhanced root growth to reach water in deeper soil layers, reduced shoot growth to preserve limited resources) enabling the plant to survive drought. In addition to AtMYB2, there may be other, as-yet-unknown transcription factors (TFs) involved in the downregulation of CK biosynthesis and signaling through ABA-dependent or -independent pathways. AHK, *Arabidopsis* receptor histidine kinase; AHP, *Arabidopsis* histidine phosphotransfer protein; ARR, *Arabidopsis* response regulator; CKX, CK oxidase/dehydrogenase; IPT, isopentenyltransferase; AtMYB2, R2R3-type MYB (myeloblastosis) TF in *Arabidopsis thaliana*.

Regarding the negative effect of CKs on drought tolerance, studies of drought responses using *Arabidopsis* CK-deficient mutants, such as *CKX1*, *CKX2*, *CKX3*, or *CKX4* overexpressors or *ipt1/3/5/7* mutants, or CK-signaling mutants, such as *ahk2/3*, *ahp2/3/5*, or *arr1/10/12* plants, have provided evidence that CKs, and thus CK signaling, can act as negative regulators of plant drought adaptation [5–7]. Furthermore, the results of these studies have shown an emerging and complex picture of a CK-controlled network underlying drought responses in a yin-and-yang manner; that is, suppressing an important hormonal pathway, which causes a decrease in shoot:root ratio (reduced shoot growth but enhanced root growth), for acclimation and better adaptation to stress. These findings collectively indicate that mechanisms exist in plants, with the involvement of at least ABA, that trigger repression of CK biosynthesis and signaling under drought, leading to a series of physiological and biochemical changes and thereby physiological and morphological adjustments for plant survival [6,8]. As shown in Figure 1, our current understanding in *Arabidopsis* suggests that on drought exposure plants induce biosynthesis of ABA, whose accumulation activates *AtMYB2* [9]. The AtMYB2 TF subsequently downregulates several *IPT* genes–namely, *IPT1*, *4*, *5*, *6*, and *8*–resulting in a reduction in endogenous CK levels [10] and, as a consequence, suppression of CK signaling. In addition to AtMYB2, other, unknown drought- and/or ABA-responsive TFs might also downregulate *IPT* or upregulate *CKX* genes, altering CK homeostasis under stress. Drought was also shown to suppress the expression of several components of CK signaling that act as negative regulators of plant drought adaptation (e.g., *AHP2*, *AHP3*, *AHP5*, *ARR1*, *ARR10*, *ARR12*) through as-yet-unknown TFs. This sophisticated transcriptional regulation attenuates the inhibitory effects of CK signaling on the expression of stress- and/or ABA-responsive genes, leading to plant survival [7,8].

Hence, CK metabolism is regulated in a highly dynamic way in response to water deficit [1,6]. A reduction in CK levels under drought results in reduced shoot growth to save limited resources as well as appropriate reallocation of resources to promote the growth of the root system to access water in deeper soil layers [5]. Furthermore, suppression of CK signaling to a certain level also leads to reduced stomatal aperture, enhancement of anthocyanin biosynthesis and thus the antioxidant system, and the maintenance of cell membrane integrity and potential accumulation of osmolytes, which together enable plants to reduce cellular dehydration and decrease drought-induced oxidative stress [6–8,11] (Figure 1). Understanding this yin–yang relationship between CK homeostasis and plant acclimation and/or adaptation to drought may help us establish a promising strategy for the development of improved drought-tolerant crops through preprogrammed reduction of endogenous CK levels using a root-specific or stress-inducible promoter. A recent success in the development of transgenic barley (*Hordeum vulgare*) using root-specific overexpression of the *Arabidopsis CKX1* gene is encouraging evidence for scientists in this research field [12].

Concluding Remarks

Recent findings provide compelling evidence that, to cope with drought, plants at certain times of stress can activate acclimatizing mechanisms to repress CK signaling by reducing CK biosynthesis and downregulating the expression of certain components of CK signaling. This in turns leads to morphological and physiological adjustments that allow plants to survive water deficit. The identification and dissection of all of the components involved in this regulatory network are of high importance and remain a task for the future (Figure 1) requiring a concerted effort by the plant research community.

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