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Molecular Dynamics of Cytokinin Signaling Pathways in Plant Growth, Development, and Stress Responses: An Integrative Review

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Abstract

Cytokinins (CKs) are essential phytohormones regulating plant growth, development, and adaptive responses to environmental stress. Over the past two decades, substantial progress has been made in understanding cytokinin perception, signal transduction, receptor-mediated control of plant morphology, and their interactions with other hormonal and stress-response pathways. This review synthesizes findings from foundational and contemporary studies to provide a comprehensive overview of cytokinin signaling, including histidine kinase receptors, multi-step phosphorelay pathways, cytokinin response factors (CRFs), and downstream transcriptional networks. The roles of cytokinin signaling in shoot and root growth, leaf senescence, seed germination, organogenesis, and metabolism are explored with reference to key genetic studies in Arabidopsis and maize. Moreover, the review examines the molecular mechanisms of cytokinin-mediated abiotic and biotic stress responses, with particular emphasis on receptor AHK1/ATHK1 function in drought and salinity signaling, and hormone cross-talk during stress adaptation. Current research trends highlight the importance of integrating cytokinin networks with abscisic acid (ABA), ethylene, and other hormonal pathways to fine-tune plant physiological responses. The review concludes by discussing gaps in current knowledge, potential applications in crop improvement, and future research directions aimed at manipulating cytokinin signaling for enhanced plant productivity and resilience.

Keywords: Cytokinin signaling, Histidine kinase receptors, AHK1, AHP proteins, Type-A response regulators, Type-B response regulators, Abiotic stress signaling, Drought tolerance

1. Introduction

Plant hormones are key regulatory molecules that orchestrate cellular differentiation, organ patterning, environmental adaptation, and metabolic balance. Among these, **cytokinins (CKs)** represent a major class of adenine-derived hormones involved in diverse biological processes such as shoot development, root architecture modulation, leaf senescence suppression, nutrient allocation, and stress signaling. Since their first discovery as “kinetin,” cytokinins have become central to understanding plant development.

The advancement of molecular tools has led to remarkable progress in deciphering cytokinin signaling pathways. These pathways predominantly rely on **two-component systems**, consisting of sensor histidine kinases, histidine phosphotransfer proteins, and response regulators. Classic studies in Arabidopsis established the centrality of cytokinin receptor families AHK2, AHK3, and CRE1/AHK4 in mediating cytokinin perception and signal transduction. Functional characterization of receptor mutants has revealed roles in shoot development, leaf longevity, seed size determination, and root patterning. For example, **cytokinin receptor mutants exhibit defects in shoot growth, delayed or premature leaf senescence, impaired germination, and alterations in cytokinin metabolism** (Riefler et al., 2005).

Recent advancements have extended these findings to crop species such as rice and maize, where cytokinin receptor-like histidine kinases also influence developmental pathways and stress adaptation (Wang et al., 2014). Another significant direction in cytokinin biology relates to **crosstalk between cytokinin and other hormonal networks**, especially ABA during drought and salinity stress. Research on AHK1/ATHK1 revealed its dual role as both an osmosensor and a cytokinin-related histidine kinase involved in ABA-dependent responses (Tran et al., 2007). These discoveries highlight the complexity and versatility of cytokinin signaling.

Furthermore, recent studies emphasize the crucial role of cytokinins in **de novo shoot organogenesis**, where accurate hormonal regulation is required to achieve cellular reprogramming and meristem formation (Hnatuszko-Konka et al., 2021). Simultaneously, molecular investigations into **cytokinin response factors (CRFs)** demonstrate how transcriptional regulators integrate cytokinin signals to shape plant growth and promote senescence (Raines et al., 2015).

This review consolidates current knowledge across developmental functions, molecular mechanisms, and stress signaling roles of cytokinins, providing a holistic and updated understanding of this intricate hormonal pathway.

2. Cytokinin Perception and Signal Transduction Mechanisms

2.1 Cytokinin Receptors: Histidine Kinases (HKs)

Cytokinins are perceived by membrane-bound **histidine kinase receptors**, structurally similar to bacterial two-component signaling proteins. In Arabidopsis, **AHK2, AHK3, and CRE1/AHK4** are the primary cytokinin receptors. These receptors possess extracellular ligand-binding CHASE domains, transmembrane helices, and intracellular kinase domains.

Riefler et al. (2005) demonstrated that single receptor mutants show mild phenotypes, while **double and triple mutants lead to severe defects** including stunted shoot growth, premature leaf senescence, reduced root elongation, and impaired seed size. These findings confirmed that cytokinin perception is integral to the entire plant life cycle.

Receptor / Gene	Plant Species	Primary Biological Role	Phenotypic Effects When Mutated	Supporting Study
AHK1/ATHK1	*Arabidopsis thaliana*	Osmotic sensing, drought & salinity response	Hypersensitivity to drought & salt; altered ABA response	Tran et al. (2007)
AHK2	*Arabidopsis*	Cytokinin perception in shoots and roots	Reduced shoot growth; premature senescence	Riefler et al. (2005)
AHK3	*Arabidopsis*	Major regulator of leaf senescence	Rapid leaf yellowing, reduced longevity	Riefler et al. (2005)
CRE1/AHK4	*Arabidopsis*	Primary cytokinin receptor; regulates meristem activity	Defective shoot meristem, altered root development	Riefler et al. (2005)
ZmHK1, ZmHK2, ZmHK3	*Zea mays*	Cytokinin responsiveness in crop species	Altered growth response under cytokinin treatment	Wang et al. (2014)

Table 1. Key Cytokinin Receptors and Their Known Functions
This Table summarizes the major cytokinin receptors characterized in both *Arabidopsis* and maize. These receptors form the first layer of cytokinin perception and determine how plants translate extracellular hormone signals into developmental and physiological changes. Mutational analyses, especially in *Arabidopsis*, have been fundamental in revealing receptor functionality.

In maize, orthologous cytokinin receptor-like histidine kinases have been identified and characterized. Wang et al. (2014) showed that cytokinin receptor-like genes in maize are responsive to cytokinin treatment and can influence developmental processes, suggesting conserved receptor functionality across species.

2.2 Multi-Step Phosphorelay Signaling

Upon cytokinin binding, receptor autophosphorylation occurs on a conserved histidine residue. The phosphate group is then transferred through a stepwise series of reactions:

1. **Histidine Kinase (HK) →**
2. **Histidine Phosphotransfer proteins (AHPs) →**
3. **Type-A and Type-B Response Regulators (ARRs)**

Type-B ARRs function as **positive regulators**, activating cytokinin-responsive gene expression, while type-A ARRs act as **negative feedback regulators**, stabilizing cytokinin output.

The architecture of the multi-step phosphorelay allows flexible and modular control of cytokinin responses, enabling cross-talk with environmental signaling pathways.

Component	Type	Subcellular Localization	Functional Role	Regulation
Histidine Kinases (HKs)	Sensor proteins	Plasma membrane / ER	Cytokinin binding & autophosphorylation	Activated by CK
AHPs	Phosphotransfer proteins	Cytoplasm & nucleus	Shuttle phosphate from HKs to ARRs	Regulated at transcriptional level
Type-B ARRs	Transcription factors	Nucleus	Activate CK-responsive genes	Positive regulators
Type-A ARRs	Negative regulators	Nucleus	Feedback inhibition of CK signaling	Rapidly induced by CK

Table 2. Components of the Cytokinin Phosphorelay System

This table outlines the core steps of cytokinin signaling. The two-component system is conserved across plant lineages and enables flexible tuning of cytokinin responses. The interaction between Type-A and Type-B ARRs ensures precise temporal control.

2.3 Cytokinin Response Factors (CRFs)

An important branch of cytokinin signaling involves **Cytokinin Response Factors (CRFs)**. These are AP2/ERF transcription factors that modulate root and shoot development.

Raines et al. (2015) showed that CRFs regulate gene expression patterns controlling growth promotion and leaf senescence. Overexpression of CRFs leads to enhanced shoot growth but accelerates senescence, indicating the balancing role of these factors in developmental processes.

CRFs also integrate multiple hormonal signals, allowing cytokinin pathways to respond dynamically to environmental conditions.

3. Cytokinin Regulation of Plant Development

3.1 Shoot Growth and Meristem Activity

Cytokinins strongly promote shoot initiation, expansion, and meristem maintenance. Howell (2003) highlighted the importance of cytokinin signaling in establishing shoot apical meristem identity and in driving cell division in developing leaves and stems.

In Arabidopsis receptor mutants, shoot meristem activity is greatly diminished, leading to reduced rosette size and limited branching (Riefler et al., 2005). Conversely, cytokinin overproduction results in hyperproliferation of shoot tissues.

Developmental Process	Cytokinin Function	Molecular Mechanism	Key Research
Shoot apical meristem maintenance	Promotes stem cell proliferation	Activation of WUSCHEL pathway	Howell (2003)
Leaf expansion	Enhances cell division and enlargement	Receptor-mediated CK signaling	Riefler et al. (2005)
Branching	Stimulates axillary bud outgrowth	Interaction with auxin transport	Howell (2003)

Table 3. Roles of Cytokinins in Shoot Development

This table highlights the essential functions of cytokinins in shoot development. Their activity ensures sustained growth and organ formation, explaining why CK-deficient plants show dwarfism.

3.2 Root Architecture and Cytokinin-Auxin Interactions

While auxin promotes root elongation, cytokinins typically inhibit primary root growth and promote lateral root formation. This antagonistic interaction is essential for root plasticity and nutrient foraging.

AHK mutants exhibit altered root meristem size, root cap formation defects, and nutrient uptake imbalances (Riefler et al., 2005). CRF proteins modulate root elongation by linking cytokinin signaling with root developmental outcomes (Raines et al., 2015).

3.3 Leaf Senescence and Photosynthetic Regulation

CKs act as strong **anti-senescence hormones**. They delay chlorophyll degradation, maintain photosynthetic capacity, and sustain nutrient remobilization. AHK3 plays a major role in delaying senescence, as demonstrated in receptor mutant studies (Riefler et al., 2005).

In contrast, CRFs promote senescence when overexpressed, illustrating the dual regulatory roles in maintaining leaf lifespan (Raines et al., 2015).

Pathway or Factor	Effect on Senescence	Supporting Evidence
AHK3 receptor	Delays senescence	Riefler et al. (2005)
CRFs	Promote senescence when overexpressed	Raines et al. (2015)

Table 4. Cytokinin-Dependent Regulation of Leaf Senescence

While cytokinins usually delay senescence, certain downstream factors like CRFs may promote it. This dual behavior highlights the fine-tuning nature of CK signaling.

3.4 Seed Development and Germination

Cytokinins regulate seed size, dormancy, and germination efficiency. Loss of receptor function leads to smaller seeds and decreased germination rates due to altered hormonal balance (Riefler et al., 2005).

3.5 De Novo Shoot Organogenesis

De novo shoot organogenesis depends heavily on cytokinin-mediated reprogramming. Hnatuszko-Konka et al. (2021) detailed how cytokinins drive the formation of shoot meristems from differentiated tissues, emphasizing the coordinated activation of WUSCHEL-related pathways.

Cytokinin signaling determines the balance between callus formation, shoot induction, and meristem stability, making it crucial in biotechnology and plant regeneration.

4. Cytokinin-Mediated Stress Signaling

4.1 Cytokinin and Drought/Salinity Stress

A landmark study by Tran et al. (2007) highlighted the role of **AHK1/ATHK1** in osmotic stress responses. This receptor-like kinase acts as a sensor in drought and salinity signaling, linking cytokinin pathways with ABA-mediated stress tolerance.

Loss-of-function mutants showed hypersensitivity to salt and drought, while overexpression resulted in stress tolerance.

This suggests that cytokinin receptors can function independently of classical CK signaling to support environmental adaptation.

Stress Type	CK Pathway Involved	Mechanism	Key Evidence
Drought	AHK1/ATHK1 activation	ABA-linked osmotic sensing	Tran et al. (2007)
Salinity	AHK1-mediated salt tolerance	Cytokinin-controlled osmoprotective genes	Tran et al. (2007)
Heat	Reduced CK levels	Promotes stress avoidance	O'Brien & Benková (2013)
Nutrient deprivation	CK downregulation	Adjusts root architecture	Howell (2003)

Table 5. Cytokinin Signaling in Abiotic Stress Responses

4.2 Crosstalk Between Cytokinin and ABA

ABA is the primary hormone for drought, salt, and desiccation stress. O'Brien & Benková (2013) explained that cytokinin–ABA interactions determine stomatal closure, water retention, and gene expression changes.

Under drought conditions:

- ABA levels **rise**,
- Cytokinin synthesis **declines**,
- Receptor activities adjust accordingly.

This antagonistic dynamic ensures energy conservation and survival under extreme conditions.

4.3 Cytokinin and Biotic Stress Responses

Cytokinins modulate pathogen resistance through metabolic adjustments and hormonal interactions. Crosstalk with SA, JA, and ethylene determines immune responses (O'Brien & Benková, 2013).

CKs can either enhance or suppress immunity depending on tissue type, receptor expression, and microbial interactions.

5. Crosstalk Between Cytokinin and Other Hormonal Pathways

5.1 Cytokinin–Auxin Interactions

CRFs serve as nodes integrating auxin and cytokinin, especially in root development and organogenesis (Raines et al., 2015). Auxin induces cytokinin biosynthesis in shoots, while cytokinin modulates auxin transport through PIN protein regulation.

5.2 Cytokinin–Ethylene Interactions

Ethylene and cytokinins share interconnected roles in growth inhibition, senescence, and stress adaptation. Ethylene can enhance cytokinin degradation, while cytokinins influence ethylene biosynthesis.

5.3 Cytokinin–ABA Crosstalk

As previously noted, ABA and CK interactions coordinate responses to water-deficit stress. AHK1 functions as a mediator linking hormonal signals to gene expression networks (Tran et al., 2007).

6. Cytokinin Signaling in Crop Improvement

6.1 Enhancing Stress Tolerance

AHK1-mediated pathways can be manipulated to engineer crop resilience to drought and salinity. Cytokinin–ABA balance becomes a target for genetic improvement.

6.2 Improving Yield and Biomass

Regulating receptor activity or modifying CRF gene expression can increase shoot biomass, enhance photosynthetic efficiency, and delay senescence, improving grain yield potential.

6.3 Applications in Tissue Culture

Understanding de novo organogenesis is fundamental in micropropagation and genetic engineering. Cytokinin optimization improves callus regeneration and shoot multiplication efficiency (Hnatuszko-Konka et al., 2021).

7. Knowledge Gaps and Future Perspectives

Despite major advances, significant gaps remain:

1. **Precise structural understanding** of receptor–cytokinin interactions.
2. **Systems-level models** integrating cytokinin with all hormonal pathways.
3. **Species-specific signaling variations**, especially in non-model plants.

4. **Engineering strategies** for fine-tuning cytokinin levels without negative trade-offs.
5. **Role of CRFs in environmental signaling** and how they coordinate multi-hormonal networks.

Future research should focus on exploiting CRISPR-based editing of cytokinin receptor genes, targeted modification of phosphorelay components, and designing synthetic hormone analogues to modulate plant development and stress tolerance.

8. Conclusion

Cytokinin signaling represents a central regulatory framework that profoundly shapes plant growth, developmental patterning, and responses to environmental challenges. Through its network of membrane-localized receptors, multi-step phosphorelay elements, and transcriptional regulators such as CRFs, cytokinin activity governs a wide spectrum of physiological processes—from shoot morphogenesis and nutrient allocation to stress adaptation and regenerative capacity.

The growing body of research has revealed cytokinin pathways to be highly dynamic, integrating internal developmental cues with external environmental signals. Their interaction with other hormonal systems, including auxin, ethylene, and abscisic acid, forms an intricate crosstalk that fine-tunes plant performance under both optimal and adverse conditions. This interconnectedness highlights cytokinin signaling not as an isolated pathway but as a pivotal hub coordinating multiple layers of plant biology.

Harnessing and modifying cytokinin-mediated processes opens significant opportunities for improving crop resilience, optimizing yield, and enhancing the efficiency of tissue culture and regeneration technologies. As studies continue to uncover the diversity of receptor functions, species-specific signaling variations, and downstream transcriptional networks, a more comprehensive understanding will emerge. This knowledge will ultimately support innovative strategies for sustainable agriculture and long-term food security.

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