Cytokinins
Cytokinins

Cytokinins (CKs):
• promote cell division in the shoot
• delay leaf senescence
• regulate nutrient allocation
• promote root nodule development
• contribute to environmental signaling and pathogen responses
• regulate auxin action and distribution
Cytokinin was discovered through efforts to identify compounds that increase the growth of plant cells in culture.

In the 1950s, Folke Skoog’s research group identified a synthetic cytokinin, kinetin.

Cytokinin and auxin regulate organogenesis in tissue culture

Tobacco leaf discs are placed into sterile culture dishes on medium containing various hormones.

Images courtesy of Richard Amasino.
Skoog’s group showed that CK promotes shoot growth in culture.

They also recognized that auxin and CK act antagonistically, and that the ratio between the hormones is critical for their effects.

Cytokinins delay leaf senescence

Senescence is dramatically reduced in kinetin-treated leaves or leaves of plants producing higher levels of cytokinins.

Lecture outline

Cytokinin homeostasis

Cytokinin perception and signaling

Cytokinin’s roles in whole-plant processes

Synthesis

Conjugation

Catabolism

Transport

Perception (receptor)

TF activation/inactivation

Target genes

Biological Functions

Cytokinin homeostasis

Structure of major cytokinins

Cytokinins are N\(^6\)-substituted adenine-related compounds. *Trans*-zeatin and isopentenyl-adenine are the most active and abundant CKs.

- **Adenine**
- **Trans-zeatin (tZ)**
- **Isopentenyl-adenine (iP)**
The plant pathogen *Agrobacterium tumefaciens* induces hormone-based tumors.

*Agrobacterium tumefaciens* is a natural plant pathogen. It causes crown gall disease and tumor-like growths by inducing the plant to produce auxin and cytokinin.
**Agrobacterium tumefaciens** transfers T-DNA to plant genomes

*Agrobacterium tumefaciens* carries tumor-inducing (Ti) plasmids. A subset of the plasmid DNA called transfer-DNA (T-DNA) is mobilized into the plant nucleus.
T-DNA includes genes for biosynthesis of auxin and cytokinin
T-DNA includes genes for biosynthesis of auxin and cytokinin.

These studies showed that the *Agrobacterium* *tmr* gene encodes a cytokinin biosynthetic enzyme.
The *tmr* gene encodes isopentenyltransferase, a key enzyme in CK synthesis.

The bacterial *IPT* gene uses AMP exclusively as a substrate whereas plant *IPT* genes prefer ADP or ATP.

The bacterial *IPT* gene was used to isolate plant *IPT* genes

*AtIPT2* (and *AtIPT9*) encode tRNA-IPT enzymes that use tRNA as a substrate.

*Arabidopsis* and rice encode 7 and 8 IPTs respectively.

The iP precursors iPDP or iPTP can be *trans*-hydroxylated by CYP735A to produce tZDP or tZTP, precursors of tZ.

Therefore, CYP735A activity contributes to the relative abundance of iP vs tZ.
Production of active CKs

$$\text{ADP/ATP} + \text{DMAPP} \rightarrow \text{Isopentenyl-transferase (IPT)}$$

$$\text{iPP/iPTP} \rightarrow \text{CYP735A (trans-hydroxylation)}$$

IP and tZ are produced by dephosphorylation and deribosylation

Production of active CKs

This can occur via one or two enzymatic steps; *LONELY GUY* encode an enzyme that produces active CKs in a single step.

Altering expression of CK synthetic enzymes affects plant growth...

IPT overexpression causes reduced apical dominance, reduced root growth and delayed leaf senescence

Elevated CK promotes shoot bud outgrowth

Elevated CK promotes shoot growth and restricts root growth

Wild type  IPT overexpression

ipt mutants have reduced shoot growth and enhanced root elongation growth

A quadruple ipt loss-of-function mutant reduces CK levels to less than 20% of those in wild-type plants

These CK deficient mutants have dramatically reduced shoot apical meristems and shoot growth and enhanced root growth

CYP735A expression (and tZ production) is tightly regulated

CYP735A genes are root-specific, induced by cytokinins and repressed by auxin or ABA

Loss-of-function *log* mutants fail to maintain the shoot meristem

*LONELY GUY (LOG)* mRNA is specifically localized in shoot meristem tips. Loss-of-function *log* mutants show reduced shoot branching and abnormal flowers, presumably caused by reduced levels of active CK.

**LONELY GUY** genes contribute to CK production in *Arabidopsis*

As in rice, loss-of-function log mutants in *Arabidopsis* have smaller inflorescences and fewer flowers and seed pods.

LOG overexpression phenotypes are more subtle than *IPT*-overexpression; shown here is a delay in leaf senescence.

Cytokinin can be inactivated by conjugation or degradation

The CKX genes are important regulators of active cytokinin levels

CKX genes are CK-induced

CK biosynthesis and degradation is compartmentalized within cells

The cellular and subcellular distribution of synthetic and degradation enzymes ensures that endogenous cytokinin levels can be precisely regulated.

CKX overexpression enhances root growth and decreases shoot growth

Notice that these growth effects are very similar to those caused by the loss-of-function *ipt* mutations!

Increasing [CK] through *ckx* knock-outs leads to increased seed yields

Elevated levels of CK correlates with increased meristem size and increased numbers of seeds per plant, suggesting a non-transgenic approach to increasing crop yields.

Natural variation in CKX expression affects grain production in rice

The rice variety Koshihikari (Ko) has elevated expression of OsCKX2, lower CK levels, and reduced grain production as compared to the closely related near-inbred-line (NIL).

Rice plants that accumulate more CK can produce more grain per plant because of changes in inflorescence architecture.

CK acts as a paracrine and a long-distance signal

A localized induction of IPT promotes lateral bud outgrowth only at the induced node, indicating that the synthesized CK acts locally only (like a paracrine signal).

The log phenotype also demonstrates a requirement for localized CK synthesis.

Two non-specific cytokinin transport proteins have been identified.

PUP preferentially moves the free base, and ENT moves the riboside, but neither is specific for CKs and their biological functions are not well characterized.

Purine permease (PUP)  
Equilibrative Nucleoside Transporter (ENT)

Different CKs are produced and transported differently throughout the plant. These different forms may convey different information.

The xylem sap mostly contains tZ and tZ riboside which is synthesized at high levels in the roots by CYP735A.

CKs translocate through the xylem and phloem.
Wild-type and *ipt1;3;5;7* mutant grafts reveal CK translocation

The *ipt1;3;5;7* mutants (m) make very narrow stems with little secondary growth. This phenotype is rescued by grafting with wild-type (w) shoots or wild-type roots, demonstrating long-distance translocation of CKs.

These studies also demonstrate that CKs have a critical role in vascular cambium activity.
CKs are synthesized by IPTs, CYP735As and LOGs
CKs can be reversibly and irreversibly inactivated

Increasing CK levels:
• enhance shoot growth
• decrease apical dominance
• delay leaf senescence
• decrease root growth

CKs can act as paracrine or long-distance signals

CKs can be translocated through xylem and phloem
Cytokinin perception and signaling

Cytokinin perception and signaling

Catabolism  Conjugation

Synthesis  CK  Transport  Perception (receptor)  TF activation/inactivation  Target genes  Biological Functions
CK signaling is mediated by histidine-kinase receptors

<table>
<thead>
<tr>
<th>CK receptors</th>
<th>Related HKs</th>
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<tbody>
<tr>
<td>Cytokinin receptor (AHK4)</td>
<td>CK receptors all have a CHASE domain that binds CK</td>
</tr>
<tr>
<td>Cytokinin receptor (AHK2)</td>
<td>Related proteins include ethylene receptors and HKs without CHASE domains</td>
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<tr>
<td>Cytokinin receptor (AHK3)</td>
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Histidine Kinase domain

All these proteins are hybrid-histidine kinase proteins related to bacterial two-component signaling systems

Higher plants have \( \geq 3 \) CK receptors with slightly different properties

The receptors apparently diversified early in the evolution of higher plants

The crystal structure of AKH4 shows the basis of CK binding specificity

Two AHK4 monomers

iP in binding pocket: only active, unconjugated CKs can bind

The CK receptors are thought to mostly be embedded in the ER membrane

Cytokinin signaling is mediated by a two-component-like system

A two-component system is a short signaling pathway that moves information from an input to an output. In bacteria it usually consists of two proteins, a histidine kinase (HK) and a response regulator (RR)
Cytokinin signaling is mediated by a two-component-like system

Perception at the input domain activates the histidine kinase domain. In bacterial systems the input domain is involved in environmental sensing.
Cytokinin signaling is mediated by a two-component-like system. The phosphoryl group is relayed to an aspartate (D) on the receiver domain of the response regulator.
Cytokinin signaling is mediated by a two-component-like system

Note that this step involves a transfer of a phosphoryl group rather than another kinase step involving ATP. A two-component phosphorelay system is different from a protein kinase cascade.
Phosphorelay systems involve high-energy bonds

Protein kinase cascades usually involve transfer of phosphate from ATP to the hydroxyl group of serine, threonine or tyrosine
Phosphorelay systems involve high-energy bonds

By contrast, the phosphoramidate bond in phosphohistidine has enough energy that it can be transferred without additional energy input.
Phosphorelay systems involve high-energy bonds

Energy retained in the phosphoaspartate, is sufficient to transfer the phosphoryl group to histidine

Histidine 🔄 ATP 🔄 Phosphohistidinė 🔄 ADP 🔄 Aspartate 🔄 Phosphoaspartate

Histidine

Phosphoaspartate
CK receptors are hybrid histidine kinases that include the phospho-accepting aspartate.

Typical bacterial HK

Hybrid HK predominant in eukaryotes
Hybrid histidine kinases participate in multistep signaling

Typical bacterial HK

Histidine-containing phosphotransfer protein (HPT)

Response regulator

(In Arabidopsis the HPTs are referred to as AHPs)
The *Arabidopsis* cytokinin response pathway

- **Three CK receptors**
- **Five histidine phosphotransfer proteins (HPTs)** - In *Arabidopsis* known as AHPs
- **23 response regulators (RRs) of three types** – In *Arabidopsis* known as ARRs

Arabidopsis has three CK receptors, AHK2, 3 and 4

AHK4 was identified as a wooden-leg mutant (wol) and a cytokinin response mutant (cre1)

The wol root is truncated due to impaired differentiation in central cylinder. The cre1 mutants do not produce shoots in tissue culture

CK receptor function can be assayed in a protoplast system

Protoplasts carrying a CK-induced promoter fused to a reporter gene (LUC) can reveal enhanced CK responses
All three AHKs contribute to CK responsiveness in protoplasts

CK-induced transcriptional responses are mediated by AHK2, AHK3 or AHK4 (CRE1)

Arabidopsis CK receptors have partially redundant activities in vivo

CK response was determined by CK-induced greening and inhibition of root growth. Both responses are abolished in the triple mutant. Double mutant phenotypes are generally more severe than single mutant phenotypes.

CK receptors have distinct roles in CK responses

- Fertility, seed size
- Germination
- Cytochrome metabolism
- Cold-stress signaling

- Chlorophyll retention
- CK-induced photomorphogenesis

- Primary root elongation
- Root response to exogenous CK
- In vitro shoot regeneration

- Leaf cell formation
- Root branching

Downstream of the receptors: HPs and RRs

The receptors pass the phosphoryl groups to a histidine phosphotransfer protein (HPT or AHP*) which passes it to a response regulator (RR or ARR*). Type-B ARRs are transcription factors, whereas Type-A ARRs are inhibitors of CK signaling.

*AHP and ARR refer to the Arabidopsis proteins

Receptors relay phosphoryl groups to His phosphotransfer proteins

<table>
<thead>
<tr>
<th>Hybrid Histine Kinase</th>
<th>HPt Protein</th>
<th>Response Regulators</th>
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<tbody>
<tr>
<td>Cytokinin receptor</td>
<td>AHP1</td>
<td>Type-B ARR</td>
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<tr>
<td></td>
<td>AHP2</td>
<td>Type-A ARR</td>
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<td>AHP3</td>
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<td>AHP5</td>
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Three CK receptors

Five histidine phosphotransfer proteins (HPTs) - In *Arabidopsis* known as AHPs

23 response regulators (RRs) of three types – In *Arabidopsis* known as ARRs

HPTs shuttle between the cytoplasm and nucleus

Receptor

His phosphotransfer protein

Response regulator
HPTs / AHPs are necessary but act somewhat redundantly

Loss-of-function of one or two AHPs has no apparent effect, suggesting that they act somewhat redundantly in transducing CK signaling. A triple mutant has a short root characteristic of many mutants deficient in CK signaling.
Response regulators are positive or negative regulators of CK signaling

Response regulators (RRs) aka ARRs in Arabidopsis

Type-B ARRs are positive regulators, type-A and type-C are negative regulators

There are three types of response regulators

Arabidopsis: 10 Type-A ARRs
Arabidopsis: 2 Type-C ARRs
Arabidopsis: 11 Type-B ARRs

Type-B ARRs are transcriptional activators with C-terminal DNA-binding domains

Pseudoresponse regulators (PRRs) are related proteins but not involved in CK signaling

PRRs usually lack the conserved Asp residue in the receiver domain
ARR1, a type-B ARR, is a positive regulator of CK signaling

Overexpression of ARR1 makes tissues more sensitive to CK. The concentration of CK needed to produce green tissues is a good measure of CK sensitivity. Loss-of-function arr1 mutants are less sensitive to CK.

Type-B response regulators are partially redundant

Single loss-of-function *arr* mutants are still sensitive to CK
Double and triple mutants are impaired in CK responses

Type-B response regulators bind to a DNA cytokinin response motif

Type-B response regulators bind to a cytokinin response motif (CRM)

In the ARR6 promoter, other type-B ARRs bind to an extended CRM, and a 27 bp enhancer region is necessary for full gene activation by cytokinin

Transcriptional responses can be visualized by a reporter construct

The TCS cytokinin reporter consists of a concatamer of 24 repeats of the consensus B-type-ARR cytokinin response motif and a minimal 35S promoter to drive luciferase or GFP reporter expression.

Type-A ARRs are rapidly induced by cytokinins

Minutes after CK application

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**ARR5pro::GUS**

Control - β-tubulin

Type A ARRs are negative regulators of CK signaling

Expression of type-A ARRs interferes with CK-induced transcription in protoplasts

Overexpression of a type-A ARR interferes with shoot initiation in vitro

How do type-A ARRs interfere with CK signaling?

There are two non-mutually exclusive possibilities:

Type-A ARRs might compete with type-B ARRs for phosphoryl groups

Type-A ARRs might have other phosphorylation-specific functions

Two non-phosphorylatable type-A ARR were tested for function

Wild-type protein interferes with CK signaling when it is phosphorylated. Is this because it competes with type-B ARRs for P, or is it activated by phosphorylation?

- **Wild-type** D
  - D > A: Non-phosphorylatable
    - Changing the D to A prevents the protein from being phosphorylated
  - D > E: Non-phosphorylatable "phosphomimic"
    - Changing the D to E prevents the protein from being phosphorylated and adds a negative charge, mimicking the effect of the phosphorylation

A phosphomimic form of type-A ARR has partial function

The phosphomimic D > E mutation partially restores type-A ARR function. This result indicates that type-A ARRs are not solely competing with type-B ARRs for phosphoryl groups.

Type-A arr mutant is hypersensitive to CK
Adding back wild-type ARR5 restores resistance
No increased resistance
Partial function

Type-C ARRs form a distinct group based on sequence

Unlike type-A ARRs they are not induced by CK

Unlike type-B ARRs they do not have a DNA-binding domain

They *DO* have a strong histidine phosphatase activity

ARR22 overexpression confers a strong abnormal phenotype

This phenotype resembles a triple ahk receptor mutan.

ARR22 is only expressed in chalazal cells of the embryo, and there is no discernible loss-of-function phenotype.

What do type-C ARRs do?

Type-C ARRs may remove phosphoryl groups from the system

Histidine kinase activity adds phosphoryl groups to system

Type-C ARRs remove phosphoryl groups from the system via histidine phosphatase activity

Type-A ARRs
Putative competition between type-A and type-B ARRs

Type-B ARRs – CK-induced transcription
What are the downstream effects of the phosphorelay signaling system?

Type-B ARRs are transcriptional activators. Their targets include:
- Type-A ARRs
- CKXs
- Other transcription factors
- The Aux/IAA auxin repressor *SHY2*
What are the downstream effects of the phosphorelay signaling system?

CRFs (cytokinin-response factors) are another family of CK-induced transcription factors. CK induces nuclear localization of CRFs.

What happens downstream of the phosphorelay system?

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CRF activity depends on AHKs and AHPs but not ARRs….

CRFs interact with each other and with ARRs

AHPs can also homo- and heterodimerise among each other and ARR18 has been shown to form homodimers

Perception and signalling: Summary

CKs are perceived by hybrid histidine kinases

CK binding initiates a phosphorelay that ultimately activates type-B ARR (Response Regulator) transcription factors

CK signaling is negatively regulated by type-A and type-C ARRs

CK signaling is also mediated by CRFs (Cytokinin Response Factors)
CK action in whole-plant processes

CKs regulate
• Root vascular tissue development
• Shoot and root developmental patterning
• Nutrient uptake and allocation
• Leaf senescence
• Many other processes

Cytokinin’s roles in whole-plant processes
CKs regulate root vascular development

In the root vascular cylinder, some cells in the procambium differentiate into protoxylem cells (yellow arrows), others into metaxylem (yellow arrowheads) and others into phloem (red arrowheads).

The cell walls of protoxylem have a characteristic ringed structure not present in the metaxylem.

CKs regulate root vascular development

In the wooden leg (wol) mutant of AHK4 all the vascular cells differentiate to protoxylem

A triple mutant of type-B ARRs or \textit{ahks} causes the same phenotype.

Protoxylem differentiation causes the root to stop growing.

Overexpression of ARR22 causes a *wol*-like phenotype

These results suggest that the *wol* mutation causes a severe inhibition of CK signaling

CK prevents cells from differentiating into protoxylem

A loss-of-function *ahp6* mutant partially rescues the *wol* mutant

*AHP6* is expressed in the procambium cells destined to form protoxylem

**Diagram:**
- CK \(\rightarrow\) AHP6
- Pro-cambium \(\rightarrow\) Proto-xylem

AHP6 interferes with CK signaling to permit protoxylem development

The *wol* phenotype is MORE severe than a loss-of-function mutation

The *woodenleg* (*wol*) mutant allele is more severe than a loss-of-function *ahk4* mutation. Instead, it resembles a loss-of-function of all three CK receptors.
Why is the \textit{wol} mutation so severe?

Loss-of-function of the AHK4 receptor does not stop CK signaling. Why does the \textit{wol} mutation stop CK signaling?
The *wol* mutation has a constitutive phosphatase activity

A point mutation switches the mutant *wol* protein into a constitutive phosphatase that, like ARR22, removes phosphoryl groups from the phosphorelay system.
Studies of the wol mutant revealed that:

CK responses can be regulated temporally and spatially to specify developmental fates

Bidirectional transfer of phosphoryl groups and phosphatase activities add complexity (and additional controls) to the phosphorelay system
Cytokinins contribute to developmental patterning and meristem functions

CK promotes cell division and stem cell fate at the shoot apical meristem

CK inhibits root meristem size and cell division and promotes cell differentiation at the root apical meristem

Formation and maintenance of the shoot meristem requires cytokinin

Arabidopsis

stm1 mutant

stm1 mutant expressing IPT from STM promoter

STM → IPT → CK

The shootmeristemless1 mutant (stm1) fails to initiate a shoot apical meristem. This mutant can be rescued by CK application or IPT gene expression at the SAM. STM is a transcription factor that induces expression of an IPT gene.

CK regulates meristem size and phyllotaxy by interaction with auxin

Wild Type: alternate leaves
aberrant phyllotaxy (abph1) mutant: opposite leaves

The *ABPH1* gene encodes a type-A response regulator that inhibits meristem size in wild type maize. The *abph1* mutant has an enlarged meristem that disrupts auxin signaling and leads to incorrect leaf positioning.

CK induces the WUS transcription factor, which maintains stem cells

The site of overlap between *AHK4* expression and elevated CK levels specifies the position of *WUS* expression

CKs regulate leaf shape complexity

The control of leaf shape is dependent on *Knotted1-like homeobox* (*KNOX1*) gene function, which positively regulates CK biosynthesis. CKs can partially substitute for *KNOX1* function.

Cytokinins can directly stimulate lateral bud outgrowth, but auxins repress outgrowth by downregulating cytokinin synthesis at the node. If plants are decapitated, newly-synthesized cytokinins at the node are transported into the bud and cause its outgrowth.
Cytokinin and auxin organize the root meristem in the embryo

After division of the hypophysis cell in the embryo, cytokinin response in the upper lens-shaped cell and repression of cytokinin signaling by auxin in the basal cell are necessary for the establishment of the root pole.
Auxin and cytokinin work together to regulate root meristem function

The position of the transition zone, between cell division and differentiation, is defined by the convergence of cytokinin and auxin responses on the transcription factor SHY2.

- Cytokinin promotes cell differentiation by inhibition auxin transport and response, via SHY2.
- Auxin promotes cell division by inhibiting SHY2 and cytokinin biosynthesis.
- These two feedback loops therefore regulate the size of the root meristem by balancing cell proliferation and differentiation.

Cytokinin and auxin interact to regulate lateral root outgrowth

Cytokinin, via the CRE1 receptor, negatively controls lateral root initiation by repressing *PIN1* expression in the pericycle cells flanking the lateral root founder cells (light blue), causing an auxin response maxima (dark blue) in the lateral root founder cells.
Cytokinin interacts with several other hormones besides auxin

A. Gibberellin

B. Ethylene

C. Abscisic acid

D. Strigolactone

CKs contribute to nutrient uptake and allocation

Shoot systems are a source of sugars and primary metabolites that are distributed to nutrient sinks including flowers and fruits, roots, and young leaves.

Elevated CK levels increase expression of photosynthetic enzymes and delay leaf senescence.

Root systems take up mineral nutrients such as nitrate, sulfate and phosphate.

- NO\textsubscript{3}\textsuperscript{-}
- SO\textsubscript{4}\textsuperscript{2-}
- PO\textsubscript{4}\textsuperscript{3-}

Source
Sink

CO\textsubscript{2}
CKs contribute to nutrient uptake and allocation

Elevated levels of nitrate or phosphate increase the rate of CK synthesis, which ultimately decreases root growth rate. In turn, elevated CK represses nutrient uptake.

Model showing the role of CK and other hormones on nitrogen acquisition

Plants that express IPT under the regulation of a senescence-induced promoter (SAG) have significantly delayed leaf senescence.
The delay in senescence may be due to CK-induced invertase.

CK induces invertase expression. Plants expressing invertase under the regulation of SAG12 show a delay in senescence similar to that caused by SAG:IPT.

Cytokinin signaling genes are down-regulated during senescence

A high-resolution transcriptional profile shows that CK signaling is downregulated during leaf senescence.

CKs promote nitrogen assimilation via root development

In split root systems, CKs promote root growth in response to nitrogen demand.

CKs have important roles in the development of symbiotic nitrogen-fixing root nodules in legumes.
CKs are necessary and (sometimes) sufficient for root nodule development

CK signaling is necessary for development of symbiotic nodules, and a receptor gain-of-function mutant can produce nodules spontaneously.

Nodule formation in the absence of symbiotic bacteria in plant with gain-of-function CK-receptor.

Rhizobial infection threads, but not nodules, form in CK-receptor mutant.

CKs can negatively affect stress tolerance

Loss-of-function mutants affecting CK synthesis (atipt) or signaling (ahp) are drought tolerant


However, by delaying senescence, CKs increase drought tolerance

Drought-induced *IPT* expression confers drought tolerance

This suggests that an inducible system to boost CK levels prior to leaf senescence can contribute to drought tolerance

CKs contribute to plant defenses...

**CK enhances** *Arabidopsis* **immunity to the bacterial pathogen** *Pseudomonas syringae*

**CK receptor mutants (ahk2 ahk3) are more susceptible**

Leaves pretreated with cytokinins are more resistant to *Pseudomonas syringae* infection

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ARR2, a type-B ARR, interacts with TGA3, a salicylic acid-responsive transcription factor, to induce *PR1* and other defense-response genes

---


CKs contribute to plant defenses, but can be exploited by pathogens

Phytopathogenic bacteria can modify plant development through production of cytokinins, or cytokinins and auxin, to produce leafy galls or crown galls.

The gall tissue provides a good substrate for the bacteria.

Leafy gall produced by the bacterium *Rhodococcus fascians*

Crown gall produced by *Agrobacterium tumefaciens*
CKs contribute to plant defenses, but can be exploited by herbivores

Leaf-mining insects can maintain nutrient-rich “green islands” through CK accumulation – the CKs may be produced by the insects’ symbiotic bacteria!

The development of insect-induced galls involves cytokinins that may be produced by the insect larvae they house

There are many other processes mediated by CK. Identifying the specific genes that contribute to each of these will help us to understand the myriad roles that CK plays in coordinating plant growth.

CKs have diverse roles – from regulating vascular differentiation and meristem function to regulation of nutrient allocation and leaf senescence.

We are beginning to correlate specific genes with specific functions but there are still many unresolved questions.

CKs provide many unexploited opportunities for improving agricultural yields through increased stress tolerance and seed yields.
Ongoing investigations

How do catabolism and conjugation contribute to in vivo functions?

Why is localized CK synthesis sometimes critical and sometimes not?

What signals are carried by xylem-borne tZ versus phloem-borne iP?

Are signals from the three receptors integrated or kept separate?

How do the type-A and type-C ARRs work? What is the relationship with CRFs?

What are the target genes, and what do they do?

How do all these pieces fit together to make a functioning plant???