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REVIEW

Cytokinins, A classical multifaceted hormone in plant system

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Today, owing to the versatile functionality and physiological importance of the phytohormone cytokinin (Ck) is a major focus of attention in contemporary wide areas of plant science. Cytokinins (Cks) have implicated in diverse essential processes of plant growth and development as well as in regulation of key genes responsible for the metabolism and activities of plants. Cytokinin interact in a complex manner to control a myriad of aspects related to growth, development and differentiation and its deficiency also causes pleiotropic developmental changes such as reduced shoot and increased root growth. Cytokinin signaling involves His Kinase receptors that perceive cytokinin and transmit the signal via a multi-step phosphorelay similar to bacterial two-component signaling system. Also, this review present a scheme for homeostatic regulation of endogenous cytokinins level in terms of the described mechanism of cytokinin action including its receptors and steps involved in regulation of gene expression at the post-transcriptional level and its role in whole plant as well as cell division. In addition, we also demonstrate a wide variety of biological effects including those on gene expression, inhibition of auxin action, stimulation of cell cycle etc.

Key words: *Plant hormone, Arabidopsis thaliana, response regulator, homeostasis, kinetin and histidine kinase*

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The Cks were discovered in the course of studies aimed at identifying factors that stimulate plant cells to divide (i.e. undergo cytokinesis). Since their discovery, Cks have been shown to have effects on many other physiological and developmental processes as well, including leaf senescence (Gan and Amasino, 1996), nutrient mobilization (Roitsch and Ehness, 2000), apical dominance, the formation and activity of shoot apical meristem (Synkova *et al.* 1997), floral development (Faiss et al., 1997), the breaking of bud dormancy (Pospilova et al., 2000) and seed germination. Cks also appear to mediate many aspects of light–regulated development, including chloroplast differentiation (Wingler et al., 1998) the development of autotrophic metabolism (Chernyadev, 1993) and leaf and cotyledon expansion (Ma et al., 1998).

After a difficult and time consuming

fractionation of the heat-treated DNA, Skoog and his co-workers, Carlos Miller, identified a small molecule that in the presence of an auxin, would stimulate tobacco pith parenchyma tissue to proliferate in culture. They named this biologically active molecule as kinetin and demonstrated that it was adenine (or aminopurine) derivative, 6-furfurylaminopurine (Miller et al., 1955). Later research convincingly demonstrated that Cks are required together with other plant hormones for both cell division and oriented cell expansion (Sakamoto et al., 2001), influencing all aspects of plant development (Richards et al., 2001). Consequently, it has become difficult to unambiguously define typical ‘‘cytokinin activity’’. Cks displays morphogenic properties (Faiss et al., 1997) that are modulated by the environmental and defined by dynamic changes in its perception and signal transduction.

Given that feedback regulation of X-Ck, but not Ck content in shoot tissue, is affected in comparable branching mutants from divergent species. Studies of Foo et al. (2007) is consistent with the findings of Faiss et al. (1997) that Ck overproduction in roots is inadequate to stimulate shoot branching. However, it reveals the possibility that X-Ck may play a significant role under certain developmental or physiological conditions. Future studies should play attention to stages of auxillary bud and growth that may be receptive to Ck and other long distance signals and to the role of signal cross-talk (Beveridge et al., 2007).

In addition, grafting studies also have high highlighted that, regardless of shoot or root genotype; there are a correlation between increased shoot branching phenotypes and suppressed X-Ck. This raises the possibility that shoot with growing lateral branches are a source of the proposed feedback signal. The near-normal shoot Cks level suggest that either X-Ck contributes very little to shoot Ck pools or that shoots possess homeostatic mechanisms to maintain their Ck status. Thus disconnection between X-Ck and that tissue Ck levels indicates that processes in the shoot can have a dramatic influence on whole-plant Ck homeostasis. Such processes occur with other xylem and phloem. Such processes occur with other xylem and however, mobile hormones such as ABA (Wilkinson and Davies, 2002).

Cks are defined as compounds that have biological activities similar to those of trans-zeatin, while Kinetin is not a naturally occurring PGR, and it does not occur as a base in the DNA of any species. It is a by-product of the heat-induced degradation of the DNA, in which the deoxyribose sugar of adenosine is converted to a furfuryl ring and shifted from the 9 position of the adenosine ring. Some molecules act as cytokinin antagonists are able to block the action of Cks, and their effects may be overcome by adding more cytokinin. Even the most frequent used synthetic Cks, Benzyladenine (benzylaminopurine) (BAP), tetrahydro-pyranylbenzyladenine (THPBA) and NN1-diphenlyurea (non-amino purine with weak activity) do not completely share their mechanism of action with native cytokinin. Unlike native cytokinin (eg., zeatin) these are not the good substrates for the cytokinin-binding protein-CREi/WOL/AHK4, AHK2 and AHK3 which initiate intracellular phosphotransfer and is poorly transported by cytokinin efflux carriers (Beveridge et al., 1997a). Only dihydrozeatin, isopentyladenine, zeatinribosides, zeatinribotides and 2-methylthio-cis-ribosylzeatin, cis-or transzeatin and their riboside and ribotides are naturally found in plants and bacteria, respectively, and therefore, qualify as endogenous Cks, but their roles and mechanisms of action have not been satisfactory described (figure 1 and 2).

In 2001, Czech Republic hosted the 17th international Conference on “Plant growth Substances” and invited many brilliant scientists of plant biology. Nonetheless, even at that splendid assembly, the report “Biosynthesis and perception of Cks” by Kakimoto (2001). This conference appears to be the highest and most valuable event in area of Ck research. Also, year 2001 saw an amazing progressive year in field of Cks studies (Romanov, 2011).

Cytokinin signaling and other plant hormones

Ck are important regulators of development and environmental responses of plants that execute their action via the molecular machinery of signal perception and transduction. The characterization of the molecular mechanisms regulating hormone synthesis, signaling, and action are facilitating the modification of Ck biosynthetic pathways for the generation of transgenic crop plants with enhanced abiotic stress tolerance (Peleg and Blumwald, 2011). Since plant hormones generally are assumed to interact with specific receptors that reside either on the cell surface or within the cytoplasm. Two candidates for a cytokinin receptor have recently been identified. One of which tends to fit the steroid hormone receptor model while the other fits the membrane receptor model. It is possible, although unlikely, that both of these are cytokinin receptors. Until recently, our knowledge of how cytokinin works at the cellular and molecular levels is still quite fragmentary, significant progress has been achieved in regard to biosynthesis, metabolism, perception, and signal transduction.

A detailed loss of function mutant analysis used to study the role of three Ck receptors in development and their participation in a variety of Ck dependent processes. The general outcome is that the signal perception system is redundant, with all three receptors participating in most of the analysed reactions. However, the three receptors and their combination contribute to a different extent to different processes, such as root branching. Mutation in single receptor did not cause strong changes of short growth, indicating a high degree of redundancy of receptors functions in shoot growth regulation. Redundancy was not complete as combined loss of AHK2 and AHK3 restricted shoot growth, in particular, chlorophyll and leaf cell formation was reduced. This shows that CRE1/AHK4 alone does not support all Ck functions in the shoot, while these functions are maintained by either AHK2 or AHK3 alone. Interestingly, changed leaf size did not alter overall leaf shape and heteroblasty, indicating that these traits are regulated independent of Ck. These alterations are generally in accordance with the shoot phenotype of Ck-deficient Arabidopsis plant (Werner et al., 2003). However, an important difference is that a strong reduction of the Ck content lead to complete growth arrest of the apical shoot meristems (Werner et al., 2003), while triple receptors mutants are still able to establish and maintain a functional shoot meristems (Higuchi et al., 2004).

Moreover, Cks are plant hormones involved in regulation of diverse developmental and physiological processes in plants whose molecular mechanisms of action are being intensely researched. However, most rapid responses to Ck signals at the proteomic and phosphoproteomic levels are unknown. Cerny et al. (2010) indicate novel links between temperature and Ck signaling, and an involvement of calcium ions in Ck signaling. Most of the differentially regulated proteins and phosphoproteins are located in chloroplasts, suggesting an as yet uncharacterized direct signaling chain responsible for Ck action in chloroplasts. Finally, first insights into the degree of specificity of Ck receptors on phosphoproteomic effects are obtained from analyses of Ck action in a set of Ck receptor double mutants**.**

Despite its agronomic importance, not much is known about the factors regulating seed size and a possible role for Ck has only been reported recently (Werner et al., 2003). Contents of seed size involve control of growth in the embroyo, the surrounding triploid endosperm and the seed coat. Genetic studies have shown that maternal and non-maternal factors are involved in seed size regulation and that crosstalk occurs between maternal and zygotic tissues to coordinate seed size (Garcia et al., 2005). Genetic analysis of CK receptor mutants has indicated that the increase of triple mutant seed size does not depend on the genotype of the embroyo but rather is governed by the maternal and/or endospermal genotypes.

Cks also implicated in diverse and essential processes of plant growth and development, and key genes for the metabolism and actions of Cks have recently been identified. Cks are perceived by three histidine kinases-CRE1/WOL/AHK4, AHK2 and AHK3, which initiate intracellular phosphotransfer. The final destination of the transferred phosphoryl groups is response regulators (Kakimoto, 2003). Because, it is now evident that Cks are perceived by histidine kinases and transduced by two component signaling system. Signal-induced phosphorylation of proteins is an often used regulatory mechanism to transducer intracellular or extracellular signals. In plants or bacteria, phosphorylation on a nitrogen (N) atom of amino acid, histidine (a basic amino acid) residue is predominantly used (Klumpp and Krieglstein, 2002). This mode of signaling that uses this kind of phosphorylation has been referred to as the two component system. Now, it is well known that besides bacteria, archea, fungi and plants also have the two component system, consists of two proteins, the histidine kinase and the response regulator (RR). RR is characterized by the presence of receiver domain only (Stock et al., 2000). When histidine kinase senses a signal, the conserved histidine residue in the transmitter domain is phosphorylated. The phosphoryl group is then transferred to the conserved Asp residue of the receiver domain.

O.N. Kulaeva isolated a 67 KD protein, designated as zeatin-binding protein (ZBP) from the cytosol of young barley plants (Kulaeva *et al*. 1995). ZBP has a high affinity for zeatin and zeatin binding is highly specific. A molecular genetics approach to identifying a probable receptor was taken by Tatsuo Kakimoto (Kakimoto, 1996) and he generated dominant, gain-of function mutations in Arabidopsis that caused the mutant to be more sensitive than the wild type to endogenous Cks levels (Kakimoto, 1996). The first indication that Cks might be perceived by a two component system came from the identification of the histidine kinase CKI1 (Kakimoto, 1996). The CKI1 protein has not yet, been shown to bind Ck, but the ETR1 protein is an Et receptor and binds Et with a high affinity and specificity. The strong homology in the histidine kinase domains of these two proteins argues that the CKI1 protein will be found to bind Ck and that it is a Ck receptor. Moreover, microinjection studies suggest that the Ck receptor is located on the cell surface rather than in the cytosol.

The over expression of CKI1 in plants induced typical CK responses independently of CKs (Hwang and Sheen, 2001; Kakimoto, 1996). CkI1 is a candidate Ck receptor because it is a histidine kinase and it’s over expression caused CK responses. In addition, CKI1 is constitutively active as a histidine kinase when expressed in *E. coli* (Yamada et al., 2001), which contrasts with the activation of CRE1/WOL/AHK3 by Cks in these organisms. CKI1 is normally expressed in the female gametophyte only and the endosperm of immature seeds and CKI1-disruptants of Arabidopsis are lethal to final gametophyte (Pischke et al., 2002). Therefore, CKI1 is essential for developing of gametophytes, but its molecular function is unclear. CKI2 was also identified through activation tagging, and over expression of the cloned CKI2 induces Ck-independent callus growth. However, the molecular function of CKI2 is also unknown. Also, it is possible that the role of CKI1 is unrelated to CKs, but that over expression of CKI1 caused unexpected cross-talk with the Ck-signaling pathway, eliciting CK responses.

Moreover, forward and reverse genetics research found very significant progress in the identification of genuine Ck receptors. The responsible CRE1 gene is identical to WOL and AHK4, and codes for a histidine kinase (Ueguchi et al., 2001). The two component regulators and related proteins in Arabidopsis has been compiled (Hwang et al., 2002), but, however, CRE/WOL/AHK4, AHK2 and AHK3 and two histidine kinases (CKI1 and AHK5/CKI2) of unknown molecular function. Similarly to determine the molecular function of CRE1/WOL/AHK4, a yeast mutant with disrupted histidine kinase (Sln1) was used. Disruption of sln1 is lethal to yeast (*Saccharomyces cerevisae*) owing to the lack of phosphotransfer. When introduced into an sln1 mutant, CRE/WOL/AHK4 rescued the lethality only in the presence of Cks (Inoue et al., 2001).

Cell divisions produce root vascular initial cells files after germination are impaired and are considered as the primary defect in this mutant. Results of Riefler et al. (2006) supports a negative regulatory role for Ck in root growth regulation. Similarly, increased Ck content of receptor mutants indicates a homoeostatic control of steady state Ck levels through signaling. Together, the analysis reveals partially redundant functions of the Ck-receptors and prominent roles for the AHK2/AHK3 receptor combination in quantitative control of organ growth in plants, with opposite regulatory functions in roots and shoots. The phenotype in the root was consistent with the expression pattern of the CRE1 gene. The CRE1 message was first detected, by *in situ* RNA hybridisation, in the four inner most cells of the globular-stage embryo. From the heart stage onward, it is expressed in the procambium or the cotyledon shoulders, in prospective hypocotyls, and in embryonic roots. After germination, the message is abundant in the procambium and pericycle in the root (Mahonen et al. 2000).

Moreover, breakage of dormancy and seed germination is primarily controlled by a reversible red-light-dependent equilibrium of the photoreceptors; Phy A and phy B (Bentsink and Koornneef, 2002). One additional important factor to overcome ABA-induced dormancy and germinate is gibberelic acid (GA), which found as a consequence of light action (Yamaguchi et al., 1998). Now, the more rapid germination, increased dark germination and reduced far-red light sensitivity of Ck receptor mutant seeds prove the relevance of Ck in regulating this process. An alternative possibility to explain the dark germination of receptor mutants is an enhanced GA signaling as a consequence of reduced Ck signaling because seed treatment with GA overcomes partially the inhibition of dark germination (Koornnef et al., 1985). It is noteworthy that Ck signaling mediating receptors control seed germination. Mutation of CRE1/AHK4 caused the greatest enhancement of germination in the dark while mutation of AHK3 alone was sufficient for partial resistance to far-red light. Hypocotyl elongation is another red-light controlled process that is tested in the CK signaling mutants (Borthwick et al., 1952). Previously, it was shown that overexpression of the A-type ARR4 gene and insertional mutantion of A-type ARR genes (To et al., 2004). Alter redlight but not far-red light sensitivity of the hypocotyls. Neither study showed whether A-type ARR proteins play a role in redlight signaling independent of their function in Ck signaling or whether Ck and red-light signaling are functionally linked. The hypocotyls elongation assay, the receptor triple mutant reacted under all light conditions similar to the wild type. Above studies indicating that regulation of phy-B by ARR4 may be Ck dependent.

In Arabidopsis, the formation of lateral roots occurs through pericycle cells. A long arrays of hormones (e.g., Auxins, ethylene (ET), BRs, and ABA etc) as well as different mutants (e.g., nitrate, phosphate, sulphate and iron) regulate lateral root formation (Bao et al., 2004; Lopez-Bucio et al., 2003). A critical event in lateral root formation is re-entry of differentiated pericycle cells into the cell cycle and initiation of the root developmental program. Consistent with a function of Cks in precursor's cells of lateral root is the observation that initiation of lateral roots is associated with a localized repression of a Ck-responsive reporter gene, indicating spatial and temporal regulation of the Ck status during lateral root formation (Lohar et al., 2004). Riefler et al. (2006) hypothesize that physiological levels of Cks are super optimal for maximal root elongation and initiation of the lateral root formation program and they also reported that optimal conditions may be achieved by decreasing the endogenous Cks content or by decreasing Ck signaling. An important observation is that reduced Ck signaling led to an increase of the Ck content, in particular when AHK3 was mutated. Although, the increase in Ck content is apparently not sufficient to compensate for the loss of receptor activity, it indicates the existence of homeostatic content mechanisms.

Cytokinin homoeostasis:

Cytokinin activity has been established, demonstrating a wide variety of biological effects, including those on gene expression, inhibition of auxin action, stimulating of calcium flux the cell cycle, and as an anti-stress and anti-ageing compound. The influence of Ck on the chlorophyll content of leaves and their ability to retard leaf senescence was described soon after their discovery (Richmond and Lang, 1957). Several mutations in specific Ck receptors cause a reduction of the leaf chlorophyll content. Riefler et al. (2006) investigated the participation of different receptors in mediating chlorophyll retention by exogenous Ck using dark-treatment of detached leaves. Dark treatment of leaves causes so called dark-induced senescence, while mimics partially natural senescence, including chlorophyll degradation (Buchanan-Wollaston et al., 2005) and they revealed a major concentration of AHK3 in mediating Ck-dependent chlorophyll retardation in leaves.

Higher concentration of Ck induces some characteristics of light-grown plants in dark-grown wild type seedlings, such as inhibition of hypocotyls inhibition and development of leaves (Chory et al., 1994). To investigate which receptors participate in mediating this response, wild type and mutants seeds are grown in the dark on media supplemented with different concentrations of Ck. Along the result of Ricfler et al (2006) conclude that AHK3 in concentration with either AHK2 or CRE1/AHK4 is important to mediate Ck-dependent deterioration.

Recently, there are new data, which show that it occurs in cellular DNA as the product of oxidative, secondary modification and a secondary reaction of DNA. Also, new results on biochemical function of kinetin have been reported. Besides this, phytohormones, including auxins, ABA, brassinosteroids, Cks, ET, GA, and jasmonates, are involved in all aspects of plant growth, and developmental processes as well as environmental responses. However, our understanding of hormonal homeostasis is far from complete. Phytohormone conjugation is considered as a part of the mechanism to control cellular levels of these compounds. Active phytohormones are changed into multiple forms by acylation, esterification or glycosylation, for example. It is also believed that conjugation serves functions, such as irreversible inactivation, transport, compartmentalization, and protection against degradation (Piotrowska et al., 2011). Frйbort et al. (2011) summarizes the knowledge on enzymes that synthesize Cks, form Ck conjugates, and carry out irreversible elimination of the hormones, including their phylogenetic analysis and possible variations in different organisms (figure 3).

It is clear that homoeostasis of free Ck pools in the plant plays a vital role in regulating Ck action in development and plant responses to environmental stresses. The endogenous Ck-transzeatin contributes significantly to this free and hence, active pool of other kinetins. It remains to be tested whether this applies also for the various endogenous Ck. Alternatively, BAP and kinetin seems to play a specialized role during the development of seeds and fruits in a wide range of species whilst naturally occurring endogenous possibly acts in co-ordination with kinetin and BAP during plant interactions with soil microorganisms. It is likely that with more studies of kinetin, BAP and other trans-zeatin, perhaps some other specific responses will be linked to each of these compounds and that their targets receptors will be identified.

An electrochemical biosensor for detection of the plant hormone Ck is introduced. Ck homeostasis in tissues of many lower and higher plants is controlled largely by the activity of Ck dehydrogenase (CKX, EC 1.5.99.12) that catalyzes an irreversible cleavage of N^6-side chain of Cks. As shown in this review, the so called endogenous Ck form a group of compounds without a clear unifying mechanism of action. Because of the presence of different Ck like kinetin (N6-furfuryl-adenine) and BAP (both are example of a synthetic N6-substituted aminopurine cytokinin), in the model plant *Arabidopsis thaliana*, we know much more about these two compounds than we do about the different other endogenous Ck, trans-zeatin (principal cytokinin) cis-zeatin, dihydrozeatin, i6Ade and ribosyl-zeatin etc which have been detected or not in Arabidopsis and several other plants. The mechanisms of Ck action is complex and includes both fast effects occurring without involvement of gene expression and rather slower effects requiring gene expression. Cks were the least understood plant hormones in concerned to biosynthesis, metabolism, perception and signal transduction. Ck are perceived by three histidine kinases-CRE1/WOL/AHK4HK2 and AHK3 which initiate intracellular phosphotransfer, but there are the some rare data indicating that the action of the other endogenous Ck operate through this mode of action.

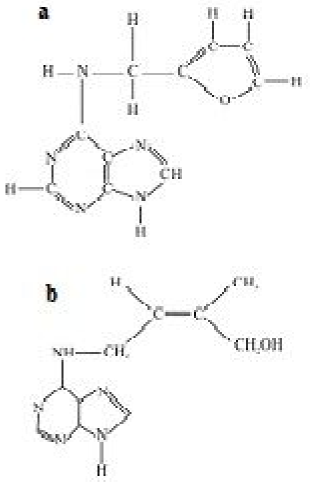

Figure 1 Molecular structures of some naturally occurring Cks. (a) kinetin (b) cis-zeatin. Free Cks also include the ribosides and ribotides of zeatin. Although they may be active as Cks by conversion to the respective bases.

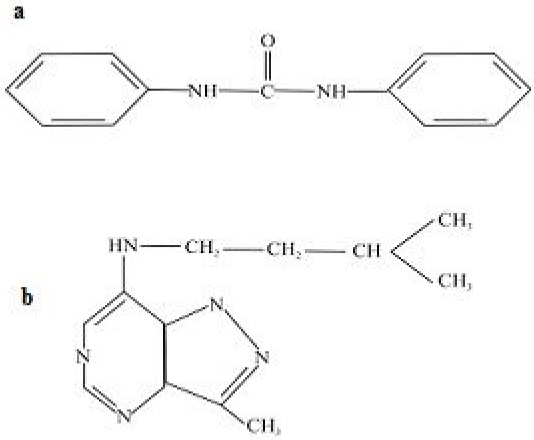

Figure 2 Molecular structures of some important synthetic Cks. (a) N-N1-diphenylurea (b) 3-methyl-7-(3-methylbytylamine pyrazolo [4, 3-D] pyrimidine.

Endogenous forms of cytokinins

Naturally occurring Ck are N6-substituted adenine derivatives. In addition to higher plants, several bacteria, including *Agrobacterium species* produce Ck (Gaudin et al., 1994). Isopentenyladenosine 5^1-monophosphate is the precursor of all other forms of Ck. Through hydroxylation of these isopentenyl side chain and reduction of the double bond, the ribotides of zeatin and dihydrozeatin are formed. It is generally thought that the free bases such as isopentenyl adenine, zeatin and dihydrozeatin are active forms of Ck. Ck with a hydroxylated side chain can be glycosylated to form the O-glucoside or O-xyloside. These reactions are reversible because O-glycosylated Ck have biological activity. Zeatin O-xylosyl transferase has been isolated from an embryos. The enzyme is predominantly localized in the endosperm (Martin et al., 1993). Because of the effect of Ck treatment may depend on both the content of endogenous Ck and the ratio between Ck and ABA. ABA is one of the hormones known to accelerate plant senescence (Yang et al., 2002). Antagonism in the impacts of ABA and Ck depend on the ratio between the concentrations of these two hormones.

Moreover, the abundance of active Ck can be reduced in two ways; through oxidative breakdown; or via conjugation of glucose to either nitrogen atoms of the adenine-ring or the free hydroxyl group of zeatin-type Ck. Ck catabolism is mediated by the enzyme Ck-oxidase. Neverthless, Ck are inactivated irreversively by two different reactions: formation of N-conjugates with glucose at the 7 or 9-positions or with alanine at the 9-position and the oxidative cleavage of the N^6-side chain of the Ck-substrate by Ck-oxidase. The substrate for Ck-oxidase is isopentenyladenine, zeatin and their ribosides. By contrast, dihydrozeatin is resistant to Ck-oxidase. Tobacco plants transformed with the IPT gene, which have an elevated Ck level, exhibit an increase in Ck-oxidase activity in both leaves and roots (Motyka et al., 1996). Genetic manipulation of Ck-oxidase may provide a strategy through which Ck level can be modified. They are required to maintain the cell division cycle but might also be involved in promoting the cell division cycle transition from undifferentiated stem cells to differentiation. Earlier work has shown that in unorganized growing cells, Ck induce the formation of shoot meristems, demonstrating that they have a function beyond maintaining the cell cycle (Skoog and Miller, 1957) (figure 4).

Cytokinin as plant growth regulators

Cks are plant growth promoting hormones involved in the specification of embryonic cells, maintenance of meristematic cells, shoot formation and development of vasculature. Cks have also emerged as a major factor in plant-microbe interactions during nodule organogenesis and pathogenesis. Microbe-originated Cks confer abnormal hypersensitivity of Cks to plants, augmenting the sink activity of infected regions. However, recent findings of Choi et al. (2011) have shed light on a distinct role of Cks in plant immune responses. They suggest that plant-borne Cks systemically induce resistance against pathogen infection which is orchestrated by endogenous Ck and salicylic acid (SA) signaling. Numerous reports ascribe a stimulatory or inhibitory function to Ck in different developmental processes such as root growth and branching, control of apical dominance in the shoot, chloroplast development, and leaf senescence. Conclusions about the biological functions of Ck have mainly been derived from studies on the consequences of exogenous Ck application or endogenously enhanced Ck levels, up to now, it has not been possible to address the reverse question: what are the consequences for plant growth and development if the endogenous Ck concentration is decreased.

Ck function as a regulatory factor in leaf cell formation is supported by the fact that transgenic Arabidopsis plants with an enhanced Ck content produced more leaf cells than control plants (Rupp et al., 1999). Further, Ck appear to restrict leaf cell size as the cells of transgenic leaves are larger than in control plants. Alternatively, a compensatory mechanism may be activated in transgenic plants to reach a genetically determined organ size, as has been reported for plants expressing dominant-negative forms of cdc2 (Hemerly et al., 1995). This suggests that the role of Ck in the regulation of development of reproductive organs might be less important than it is during the vegetative phase. It may be that once the plant has entered the reproductive cycle, a more stringent mechanism operates in the meristem to ensure the proper course of the developmental programme.

Unlike other plant hormone such as ABA, GAs and ET, no Ck biosynthetic mutants have been isolated**.**

Since previous studies used different plant species and different conditions of growth and treatment, it is difficult to compare the results obtained. Moreover, although effects in transcript accumulation in chloroplasts have been reported, the influence of Cks on plastids transcription per se has not been demonstrated yet. Shoot branching is one of the most important determinants of plant architecture and is highly responsive to environmental and endogenous cues. Long-distance signaling is essential for the regulation of auxillary shoot branching as it enables co-ordinated development of distant meristems (Dun et al., 2006). Ck can influence shoot branching but real mechanism behind this induction is unclear.

Moreover, Hartmann et al. (2010) established an *in vitro* assay using excised tuber buds to study the dormancy-releasing capacity of GA and CK and show that application of GA_3 is sufficient to induce sprouting. GA_3-treated wild-type and CKX-expressing tuber buds were subjected to a transcriptome analysis that revealed transcriptional changes in several functional groups, including cell wall metabolism, cell cycle, and auxin and ET signaling, denoting events associated with the reactivation of dormant meristems. Vercruyssen et al. (2011) results show new interactions and contribute to the molecular and physiological understanding of biomass production at the whole plant level. In addition, they also help in delay senescence or the ageing of tissues are responsible for mediating auxin transport throughout the plant and affect internodal length and leaf growth. They have a highly synergistic effect in concert with auxins and the ratios of the two groups of plant hormones affect most major growth periods during a plant’s life time. Over the past few years, exciting progress has been made to reveal the molecular mechanisms underlying the auxin-Ck action and interaction. Moreover, Su et al. (2011) briefly discuss the major progress made in Ck transport and signaling. Further, this study also suggest the complicated interaction of these two hormones in the control of shoot apical meristem and root apical meristem formation as well as their roles in vitro organ regeneration. It has been known for many decades that auxin inhibits the activation of axillary buds, and hence shoot branching, while Ck has the opposite effect.

Mьller and Leyser (2011) review the evidence for various hypotheses that have been put forward to explain how auxin and Ck influence axillary bud activity and discuss the activity the roles of auxin and Ck in regulating each other's synthesis, the cell cycle, meristem function and auxin transport, each of which could affect branching. Plant root development is mediated by the concerted action of the auxin and Ck phytohormones, with Ck serving as an antagonist of auxin transport. Similarly, Zheng et al. (2011) identify the AUXIN UP-REGULATED F-BOX PROTEIN1 (AUF1) and its potential paralog AUF2 as important positive modifiers of root elongation that tether auxin movements to Ck signaling in Arabidopsis (*Arabidopsis thaliana*). auf1 roots are also hypersensitive to Ck and have increased expression of several components of Ck signaling. Kinematic analyses of root growth and localization of the cyclin B mitotic marker showed that AUF1 does not affect root cell division but promotes Ck-mediated cell expansion in the elongation/differentiation zone. In addition, Ck counter the apical dominance introduced by auxins; they in conjugation with ET promote abscission of leaves, flower parts and fruits. In addition, Kushwah et al. (2011) results show that asymmetrical exposure of Ck at the root tip in Arabidopsis

(*Arabidopsis thaliana*) promotes cell elongation that is potentiated by glucose in a hexokinase-influenced, G-protein-independent manner.

In fact, Ck are considered the most important senescence-retarding hormones (Faiss et al., 1997), and their exogenous application has been demonstrated to prevent the degradation of chlorophyll and photosynthetic proteins (Wingler et al., 1998), to cause induction of flower or pod set (Ma et al., 1998), to reverse leaf and fruit abscission, to release dormancy (Pospisilova et al., 2000), and to modify substantially plant responses to a variety of environmental stresses, anthocyanin production and maintenance of source-sink relationship (Hutchinson and Kieber, 2002), leaf area expansion (Shah, 2008), dry matter production (Davies, 1995) and have a direct effect on determining photosynthetic parameters (Synkova et al., 1997). The relationship between auxin and Ck has long been recognized as central to normal plant growth and development (Rashotte et al., 2005).

Exogenously applied Cks delay senescence of detached leaves and keep chloroplasts photosynthetically active longer than in control leaves not treated with Cks (Romako et al., 1969). They affect chloroplast and etioplast ultra Str, chloroplast enzyme activities, pigment accumulation and the rate of photosynthesis (Yaronskaya et al., 2006). Chloroplast has enzymes for the biosynthesis of Ck and contains a set of natural Cks, including free bases, ribosides, ribotides and N-glucosides (Benkova et al., 1999; Polanska et al., 2007). The development and/or metabolic state of plastids influence the response the leaves to enzyme Ck (Kulaeva et al., 2002). Although, it is obvious that chloroplasts are among the targets of Ck action, it is not understood how Cks exerts their effects on plastids/chloroplasts. At least in part, chloroplast responses to CK may result for hormones effects on the expression of nuclear genes encoding chloroplast proteins (Chory et al., 1994; Kusnetsov et al., 1994; Kiba et al., 2005). Data of Borner (2008) showed a Ck induced stimulation of chloroplast gene transcription that depended on light and the age of leaves and cells.

Jones et al. (2010) was the first to demonstrate that Ck are biosynthesized in both aerial and root tissues and that young, developing leaves have the highest Ck biosynthetic capacity. Similarly, Tanaka et al. (2006) subsequently showed a similar phenomena exists in pea (*Pisum sativum*) in which apical dominance is maintained at least partially by auxin induced down-regulation of Ck biosynthesis in the stem. Moreover, Ck stimulates chloroplast biosynthesis and chlorophyll synthesis (Mok, 1994) and increases the photosynthetic rate. Genome-wide transcript profiling in Arabidopsis revealed that genes of photosynthesis are over represented among the genes up-regulated by Ck and several photosynthesis related genes of the chloroplast genome are also induced (Brenner et al., 2005). Similarly, the results of Werner et al. (2008) also strongly support a function of Ck in regulating shoot sink strength and its reduction may be a cause of the altered shoot phenotype. Roots of Ck-deficient plants contained less sugar compound with wild type. However, this did not negatively affect glycolysis, ATP content, or root development.

Recently genes for Ck oxidases (CKKs) have been used to decrease endogenous Ck levels in tobacco (Schmulling, 2002). Since, as already stated that Cks are a class of plant specific hormones that play a central role during the cell cycle and influence numerous developmental programmes. Ck-deficient plants developed stunted shoots with smaller apical meristem. Studies of Werner et al. (2001) suggest that Cks are an important regulatory factor of plant meristem activity and morphogenesis, with opposing roles in shoots and roots. In addition, the hypothesis predicted that Ck, together with auxin, plays an essential role in plant morphogenesis, having a profound influence on the formation of roots and shoots and their relative growth. Cell division activity is also important factor determining sink strength. Chloroplast harbour enzymes for the biosynthesis of Cks and contain a set of natural Cks, including free bases, ribosides, ribitides and N-glucosides (Polanska et al. 2007). However, most of these results have been obtained in cell culture systems and it is unclear till date that at which of the cell cycle stages Ck exerts its regulatory functions during different developmental process in different tissues.

Precursors and storage forms of Cks:

Many chemical compounds have been synthesized and tested for Ck activity. Analysis of these compounds provides insight in to the structural requirements for activity. Nearly all compounds active as Cks are N^6-substituted amino-purines and all the naturally occurring Cks are aminopurine derivatives. The Ck, Benzylaminopurine (BAP) is an example of a synthetic N^6-substituted aminopurine Ck as in kinetin. The only exception to this generalization is certain diphenylurea derivatives are not N6-substituted aminopurines, but they appear to be active as Cks by affecting the metabolism of endogenous Cks. In several species, direct application of Ck to axillary buds promotes outgrowth (Sachs and Thimann, 1964) and endogenous Ck levels have been found to rise in and around axillary buds during growth initiation (Emery et al., 1998). Widespread, unmodified Ck bases are isopentenyladenine and trans-zetin-ribose or ribose-5-phosphate may be attached at the N^9 atom of the adenine ring to form Ck ribosides or ribotides and these also generally show Ck activity when applied to plants**.** Cks are inactivated by O-glycosylation at the terminal hydroxyl group of the zeatin-type Cks or by N-glycosylation at the N3 or N7 positions of the adenine ring. O-glycosylation is reversible and O-glycosylated Cks are regarded as a storage form. The Cks ribosides and cis-zeatin, sometimes found in abundance in plants, may also be important as stored or transportable forms. Because Cks exist in the apoplasm as well as in the cytoplasm, specific transmembrane transporters for Cks may exist. CK oxidase/dehydrogenase degrades Cks by cleaving the side chain (Bilyeu et al., 2001). Ck metabolism has been reviewed in detail (Mok and Mok, 2001). Cks occur in both free and conjugated forms (not covalently attached to any macromolecules) in plants and bacteria but also occur as modified bases in certain transfer RNA molecules of all organisms (Hall et al., 1967) (figure 4).

However, Cks are not confined to plant t-RNAs. They are part of certain t-RNAs from all organisms, from bacteria to humans. Because the effect of Ck treatment may be depend on both the content of endogenous Cks and the ratio between Cks and ABA, its antagonist in the regulation of chloroplast biosynthesis in the leaf tissues, (trans-zeatin and zeatin riboside), and ABA in the basal, middle, and apical segments of barley leaves, immediately after detachment from 9-d-old plants and after preincubation of detached leaves on water for 24 h under illumination or in darkness (Zubo et al., 2008). The ABA level also rose during preincubation in darkness, but this increase was much less pronounced than in the light. This preincubation of the leaf on water in the light enhanced the content of Cks and ABA and sharply increased the ratio of ABA to Cks in detached leaves (Zubo et al., 2008). Similarly, Iqbal et al. (2006) state that Cks are often considered ABA antagonists and auxins antagonists/synergists in various processes in plants. Seed enhancement (Seed priming) with Cks is reported to increase plant salt tolerance.

It was hypothesized that Cks could increase salt tolerance in wheat plants by interacting with other plant hormones, especially auxins and ABA. Among priming agents, kinetin was effective in increasing germination rate in the salt-tolerant and early seedling growth in the salt-stress. Kinetin-priming showed a consistent promoting effect in the field and improved growth and grain yield under salt stress while BAP-priming did not alleviate the inhibitory effects of salinity stress on the germination and early seedling growth was positively correlated with leaf IAA concentration and negatively with ABA concentration under both saline and non-saline conditions. Ck bases, when given to many plants tissues, are converted of their respective nucleotides:zeatin to zeatin ribonucleotide, I6Ade ribonucleotide and so forth. They also may be converted to their glucosides (Brzobohaty et al., 1994). However, glucosides sometimes are not readily converted to free Cks. Neverthless, glucosides have been identified that will release the Ck base from Ck glucoside conjugates. For example, the rol C gene of *Agrobacterium rhizogenes* T-DNA encodes a glucosidase that can relase free Cks (Estruch et al., 1991).

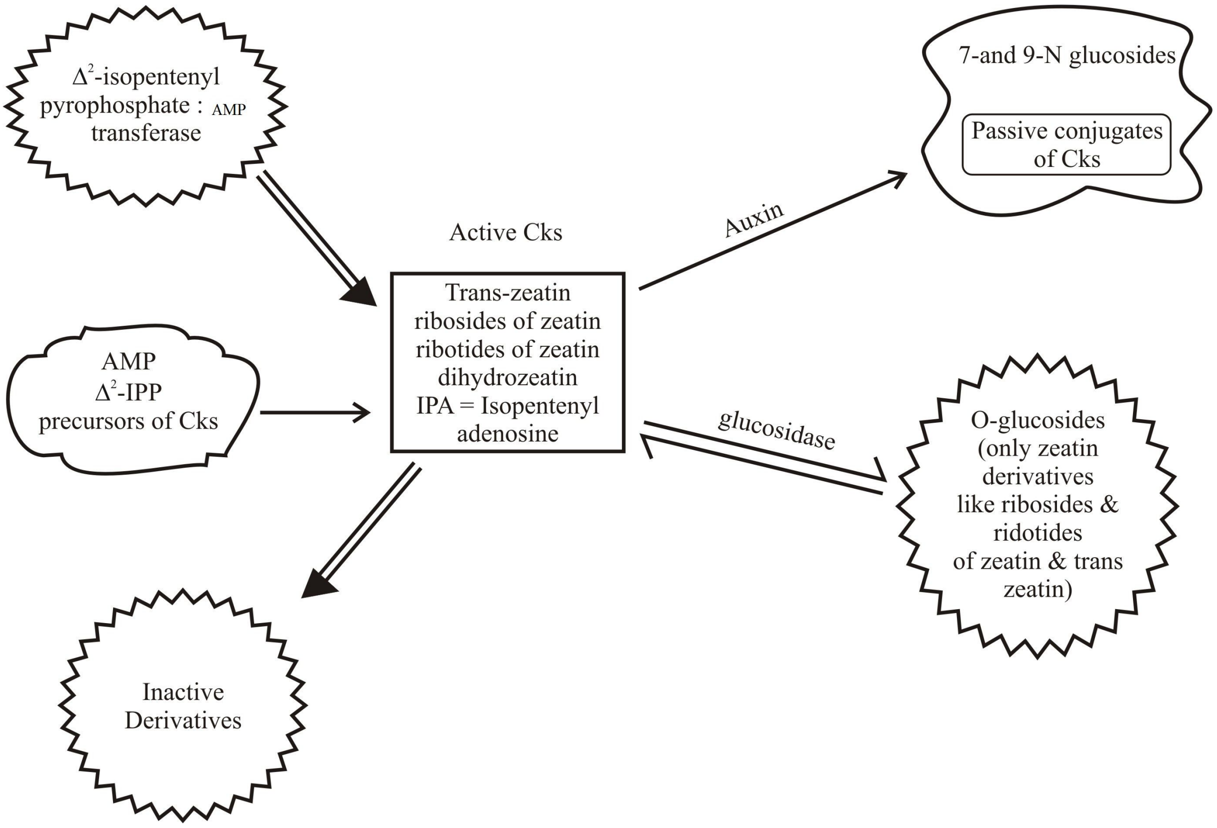

Figure 3 Potential points of control of active Cks. Generally auxin conjugates regulate enzyme activity and consequently changes in metabolite levels.

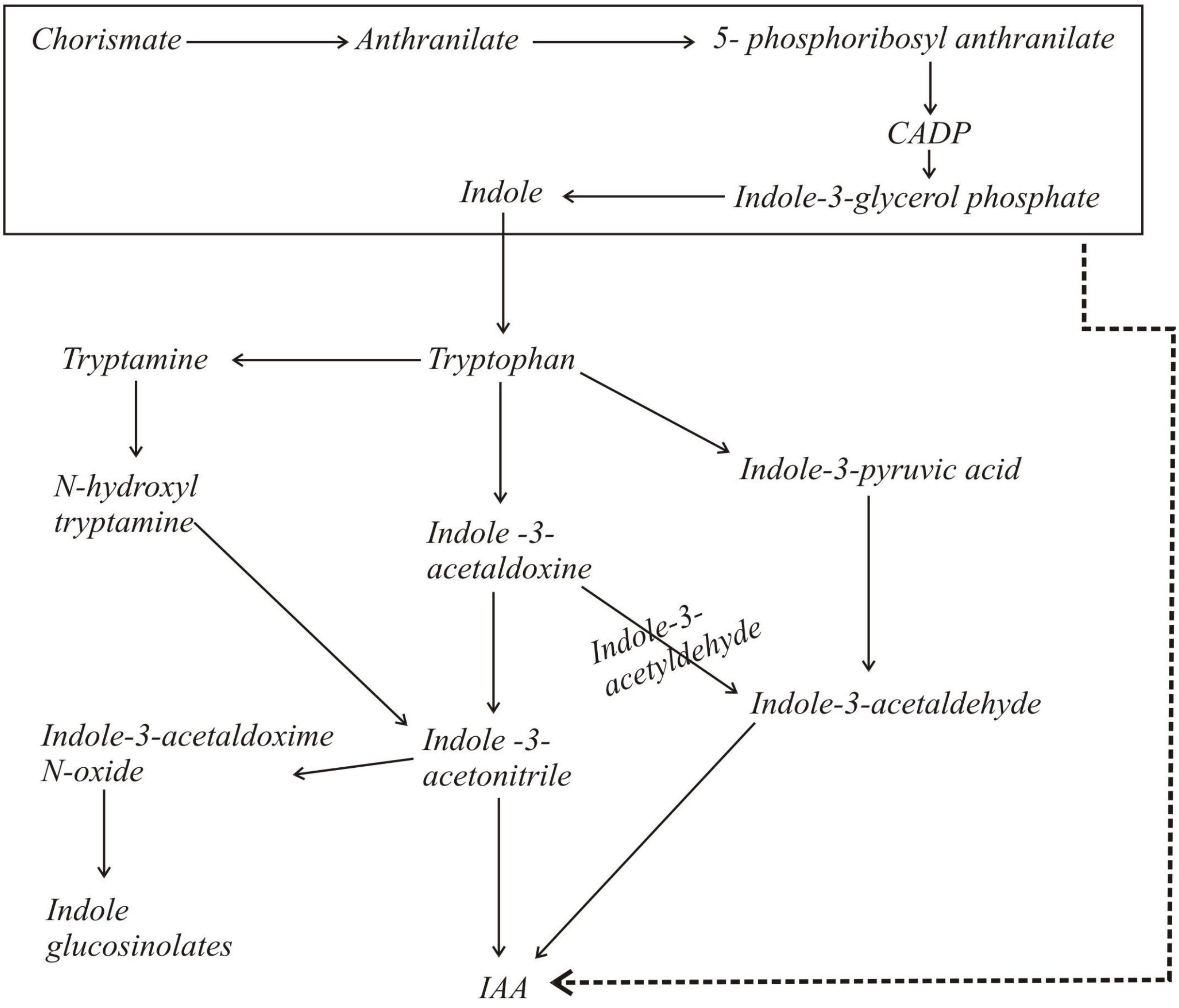

Figure 4. Biosynthetic pathway of cytokinin mediated by indole acetic acid (IAA) in plants.

Implications of cytokinins in crop physiology

The mutant analyses have yielded novel information about the involvement of different receptors and their combinations in various cytokinis-regualed processes. Many of the responses are driven by multiple cytokinin receptors in an additive manner. The contribution of a given receptor could only be identified in the absence of others. Noteworthy, mutation of AHK2 alone did not cause a significant change of Ck sensitivity in any of the tests. However, in several assays, AHK2 mutation enhanced the cytokinin resistance of AHK3 or cre1/AHK4 mutants. This indicates that AHK2 may function primarily in combination with AHK3 or CRE1/AHK4. AHK2, AHK3 receptor mutant's phenocopy to a larger extent CK-deficient plants, providing such total support for the concept of a function of ck in positive control of shoot development and negative control of root growth. Further work has to show how the Ck receptors are linked downstream of different signaling pathways in order to achieve positive or negative regulatory control on the cell cycle or exit of cells from the meristems.

Future prospective

There are a correlation found between Cks amount in a particular tissue and the native and magnitude of the stimulated responses. These displacements emerge the focus of investigations of Cks action towards mechanisms that regulate homeostasis of endogenous Cks pools in which processes such as biosynthesis, degradation, conjugation and specific transport play crucial roles, and towards mechanism of action of Cks at the cellular, molecular and genomic level. In conclusion, we would like to underline that future studies need to address the issues of relative ratio of biosynthesis degradation, import, and export of cytokinins in plant systems viz., shoot and roots. In addition, our knowledge of the Cks biosynthesis, metabolism, degradation, perception and early stages of signal transduction has increased to a greater extent. But, however, various important questions remain unsolved till date, such as we do not understand the role of Cks very well. This shortcomings has recently began to be addressed through utilizing genes for CK-oxidases to lower Cks levels; further ,more elaborate experiments using tissue-specific and inducible expression systems will definitely be informative to understand how cytokinin levels are regulated, we must uncover the regulatory mechanisms of the enzymes that catalyze biosynthesis, interconversion, and degradation of cytokinins. As for the signal response regulator loop is largely unknown.

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