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Phytochrome and cytokinin responses

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Abstract

Cytokinins (CKs) and light can elicit similar morphogenic and biochemical responses in a wide range of plant species. Contradictory reports have been presented that CKs and phytochrome may have independent or identical mechanisms of action in photomorphogenic processes. These reports, relating to seed dormancy and germination, seedling development and growth efficiency, pigment production, and the photoperiodic control of flowering are reviewed. Based on historical data and recent genetic approaches using *Arabidopsis* mutants, the possible role of CKs in physiological and biochemical pathways affected by light are discussed briefly. Together with the phytochrome system, CKs may contribute towards entrainment of circadian rhythms and thus participate in photoperiodic signalling. Both light and CKs apparently also participate in nutrient assessment pathways. Current models propose that light and CKs might act independently or sequentially through common signal transduction intermediates to control the same downstream responses. We presently have a poor understanding of the mechanism(s) whereby these signals are integrated at the molecular level and the physiological significance of the apparent overlap between the actions of phytochrome and CK cannot yet be fully appreciated.

Abbreviations: ABA = abscisic acid; ALA = 5-aminolevulinic acid; As = anthocyanin synthase; BA = N^6-benzyladenine; BR = brassinosteroid; CAB = chlorophyll *a/b* binding protein; CaM = calmodulin; cGMP = cyclic guanosine 5′-phosphate; CHI = chalcone isomerase; CHS = chalcone synthase; CK = cytokinin; cv(s) = cultivar(s); DFR = dihydroflavonol reductase; DHZ = dihydrozeatin; FNR = ferredoxin $NADP^+$ oxidoreductase; FR = far-red light; GA = gibberellin; GUS = β-glucuronidase; HIR = high irradiance response; iP = N^6-(Δ^2-isopentenyl)adenine; KIN = kinetin; LDP = long-day plant; NAA = 1-naphthaleneacetic acid; NR = nitrate reductase; PAL = phenylalanine ammonia lyase; PEPCase = phospho*enol*pyruvate carboxylase; PS = photosystem; R = red light; [9R]iP = N^6-(Δ^2-isopentenyl)adenosine; [9R]Z = Z ribonucleoside; SDP = short-day plant; Z = *trans*-zeatin

1. Introduction

Plants depend on light not only as a source of energy for photosynthesis, but also for the regulation of almost every stage of their growth and development. Although hormones are implicitly involved in these processes, to date, the molecular mechanisms by which light and plant hormones may interact to bring about these effects are largely unknown.

A general model for light signalling entails extracellular receptors initiating a signal cascade, the end result of which is either modulation of gene expression or a change in cellular physiological parameters in order to elicit a particular growth response [20]. Plants contain at least three photoreceptor systems involved in modulating growth and development, which differ in the wavelengths of light to which they are most sensitive. These are the phytochromes, blue light/UV-A

receptors and UV-B receptors. The phytochrome system senses the relative intensities of photosynthetically active red light (R) and non-photosynthetic near far-red light (FR), thus sensing information about light quality and the suitability for photosynthesis. Except for these R/FR photoreceptors, little is known about the biochemistry of light signal perception and transduction [116].

Phytochromes are homodimers located in the cytosol, their photosensory functions being based on their capacity for reversible interconversion between the R-absorbing Pr form (λ_{max} = 666–668 nm) and the FR-absorbing Pfr form (λ_{max} = 730–734 nm) when R and FR light are absorbed sequentially [25]. Photosignal perception by the receptors activates signalling pathways leading to changes in gene expression which can be translated into physiological and developmental responses to light. These responses include seed germination, seedling de-etiolation, pigment production, synthesis of a functional photosynthetic apparatus, shade avoidance and flowering [79].

Phytochrome genes encode a small family of photoreceptors. In *Arabidopsis thaliana*, the apoprotein is encoded by at least five genes, designated *PHY-A, PHY-B, PHY-C, PHY-D* and *PHY-E*. Sequences related to these genes occur in species ranging from algae to angiosperms. At least seven different *PHY* genes occur in tomato, one of which does not have a counterpart in *Arabidopsis* [114].

Phytochrome A (phyA) is necessary for continuous FR reception i.e., it mediates the FR high irradiance response (FR-HIR) and functions differently throughout the life cycle. Its conversion to the Pfr form by absorption of R causes phyA to rapidly aggregate in the cytoplasm and degrade. Phytochrome B (phyB) is necessary for continuous R perception. It is the principal phytochrome responsible for the classical R/FR reversible response and for the responses of light-grown plants to the low R/FR ratio. It is present in low levels in both the dark and light, being very stable in the Pfr form. Little is known about the *PHY-C, -D* and *-E* gene products [118]. However genetic studies, especially using mutated *Arabidopsis* plants, are now contributing much to our understanding of phytochrome functions [51].

Until the recent advent of *Arabidopsis* genetics, one of the much under-researched aspects of plant science had been the relationship between light and hormonal control of plant growth and development. This is despite the fact that plant hormones can induce germination, bolting and flowering, can affect seedling development, the photosynthetic apparatus and pigment production, can modify gene expression, and can elicit other responses identical to those initiated by phytochrome.

The close interrelationship between light and hormonal effects on plant growth and development justifiably raises the question of whether light and hormones act independently or whether the latter play an integral role as second messengers in any sequence of events initiated by physiologically active photoreceptors. Alternatively, hormones may serve as integrators of distinct signalling pathways by ‘cross-talk’, thereby influencing the capacity for transmission of light-related signals, or signalling events downstream of light perception might affect absolute concentrations of active phytohormones or the responsivity of cells to these growth regulators. Evidence that phytochrome and hormone metabolism or signal transduction are intertwined has been reviewed [24, 26, 84]. Much still needs to be learned about the intermediate steps between light perception, hormone action and physiological responses. The present review deals specifically with the possible relationships between phytochrome and CK involvement in the control of plant developmental processes.

2. Seed dormancy and germination

There are numerous reports of interactions between phytochrome and plant hormones relating to seed behaviour dating back to the early 1960s. In particular, GA was observed to act like Pfr in increasing germination, in that germination in light-sensitive seeds was usually also stimulated by GA treatment [44].

Early studies on CK stimulation of germination using lettuce seeds indicated a requirement for Pfr in order to obtain any effect [11]. Indeed, in light-requiring seeds CK treatment is generally ineffective in breaking dormancy unless some R irradiation is given or GA is also present. Thus, there appears to be a distinction between the GA responses which substitute for light in its effect on the germination of seeds in the dark and the CK effect which synergizes with light or GA to allow germination under conditions of stress such as high temperature [148].

Nevertheless, light-induced dormancy release of seeds appears to involve a CK-based component, at least in some seeds, as indicated from several studies involving the determination of endogenous CKs in seeds during dormancy break. The levels of extractable

CKs were shown to increase in lettuce fruits 24 h after exposure to light [4] and in *Rumex obtusifolius* seeds following a 10 min R treatment [157]. In *Rumex*, the effects of R were completely reversed by a subsequent exposure to FR, consistent with phytochrome control. Increases in extractable CKs have also been reported for *Apium graveolens, Picea sitchensis* and *Pinus sylvestris* [115, 143, 149]. In *A. graveolens*, the effects of R were not reversed by FR treatment and it was suggested that the reversal of R effects may not operate through CK balance but rather through some other mechanism such as an increase in germination inhibitors. Several investigations have indicated that the transient CK increases in seeds following dormancy-breaking light treatments are due to interconversion of CKs from water-soluble or storage forms to biologically active forms rather than to *de novo* synthesis [16, 149, 155, 157].

The role of specific CKs in the dormancy release of light sensitive seeds has not been elucidated. In Scots pine seeds, the content of CKs increased following R treatment, with a transient increase in the content of iP, leading the authors to suggest that this CK could be a signal transducer of active phytochrome and that its increase is the first hormonal change in the dormancy-breaking process [115]. Zeatin riboside-type compounds increased at a later step in development, around the time of radicle protrusion through the seed coat. This suggestion that different CKs may be involved at the various stages between dormancy break and germination *per se* is supported by results from osmotic priming studies with celery seeds [147]. Specificity of action of applied CKs in breaking seed dormancy was demonstrated in celery seeds, with neither Z nor [9R]Z being effective in this system [7]. Studies on the endogenous levels of CKs in celery following R treatment indicated that the major changes occurred in compounds with chromatographic properties resembling BA rather than Z and its relatives [149]. In this species, a direct relationship between the total amount of R given and the final germination value was demonstrated. This phenomenon can be partially explained in terms of the requirement for repeated renewal of Pfr for germination to take place [10] and suggests that there is a quantitative relationship between Pfr and active CK production in the seed following R treatment.

Specificity in the role of CKs in breaking seed dormancy is confounded by experiments such as those with *Spergula arvensis* [156] in which CK production appeared to be controlled by a combination of light and ethylene. Again, CK production seemed to be only part of the process initiated by light and ethylene, since CK on its own was ineffective. These data suggested that in seeds there are at least two sites of hormonal and light action, a concept supported by investigations on *Chenopodium album* seeds [76]. In this species, germination induction is primarily controlled by a high level of Pfr which can be substituted in darkness by GA at low pH and by ethylene to a lesser extent. Subsequent growth of the radicle is promoted by low Pfr and inhibited by ABA, which is in itself antagonised by GA, Z, KIN and ethylene.

In celery seeds too [148], GA action is enhanced by low pH but also by a wide range of other compounds including CKs, ethephon and fusicoccin, a compound known to stimulate proton/cation exchange across membranes in a wide range of plant tissues, including seeds. Conversely, an ionophore which has major effects on K^+ fluxes inhibited GA-induced germination but was not effective in the presence of BA. The germination of *Phacelia tanacetifolia* seeds, which are also photoblastic and thermosensitive, is characterised by the activation of K^+ uptake [106]; FR and high temperature inhibition of germination of these seeds was also overcome by fusicoccin [28]. Thus, it seems possible that the ability of phytochrome-stimulated GAs to exert their effect on germination is in some way influenced by transmembrane ion fluxes which may in turn be controlled by an interaction between CKs and ABA.

Evidence has also been presented that phytochrome effects may be related to the activity of Ca^{2+} and possibly Ca^{2+} dependent regulatory proteins, indicative of a Ca^{2+}/CaM control mechanism [28, 152]. Such results are supported by the demonstration that Ca^{2+} affects the CK/phytochrome-mediated processes of betacyanin and anthocyanin synthesis (Section 4) and is also known to be required for the synthesis and secretion of α-amylase by aleurone tissues in seeds [73]. The roles of Ca^{2+} and Ca^{2+}-activated CaM in phytochrome signal transduction [20] and CK action [61] have been reviewed. Although there appear to be close relationships between phytochrome action, phytohormones, Ca^{2+}, CaM and membrane phenomena, whether such relationships are necessarily integrated in the control of seed dormancy has not yet been identified.

One major question is why some photosensitive seeds seem to respond to GA alone, whereas others only respond partially and require other compounds to be present eg. CKs, before full germination can be

achieved in the dark, particularly at high temperatures. This was particularly apparent with celery seeds, where partial responses to GA obtained by using different cultivars could be enhanced to achieve full germination by applying BA [149]. In the most dormant cvs, full germination could never be achieved simply by increasing the GA concentration [151]. This suggests that the effects normally obtained from exposure to R require the action of a hormone complex, including GAs, CKs and other hormones. In this particular case, it seems possible that seeds of highly dormant cvs might contain higher levels of endogenous CKs than the more dormant types; unfortunately this has not been tested. However, it was demonstrated that seeds of a highly dormant cv. contained more germination inhibitors, the action of which could be overcome by CK application [150]. It has been suggested that some phytochrome effects may be mediated by tissue sensitivity to hormonal action. Tissue sensitivity itself is likely to be partly a reflection of the endogenous hormone balance within those tissues. What seems to be clear from this example of phytochrome/hormonal interaction is that attempts to relate the effects of phytochrome to a single specific hormone in any system or process may be far too simplistic.

In *Avena sativa*, the onset of phytochrome synthesis in germinating embryos is part of a rapid increase in total protein synthesis correlated with the initiation of radicle growth. Synthesis of phyA is specifically inhibited by light acting through a stable fraction of phytochrome, probably phyB [146]. Introduction of the oat *PHY-A* gene into tobacco plants, thus introducing supra-optimal levels of phyA, altered the response of seeds to FR [97]. Reduction of the fluence rate partially relieved inhibition of germination in transgenic seeds but not in those from wild-type plants. These results support the concept that the HIR is dependent on two antagonistic components, one a function of the Pfr concentration and the other a function of the phytochrome cycling rate. There is no information available at present to indicate whether the rate of CK synthesis and conversion have a major influence on one or both of these components.

Information on the exact roles of the different phytochromes in seed dormancy and germination is somewhat confusing. There are indications that seed phytochrome synthesized during seed development, mainly phyB, is responsible for the induction of germination. Evidence for this comes from the demonstration that seed of the phytochrome-deficient tomato *aurea* mutant, which lack phyA in darkness [107], can be stimulated to germinate by R treatment [52]. From studies using the de-etiolated *det1* and *det1/hy3* mutants of *Arabidopsis*, it was demonstrated that phyBfr principally controls seed germination in the dark. However, both phyA and phyB were able to promote germination in R, presumably through the action of the Pfr form [120]; phyA appeared to play only a supplementary role in the absence of phyB but could induce germination under continuous irradiation with FR [130].

Recent experiments with *Arabidopsis*, aimed at resolving these ambiguities, indicate that phyA and phyB control dormancy-break in seeds in entirely different ways [131]; phyB mediates the classical photoreversible reaction, responding to R and FR at much higher fluences than those to which phyA responds. The action of phyA is photoirreversible; it triggers seed germination in irradiation at extremely low fluence with light of within the UV-A, visible and FR range, an adaptation particularly suited for germination under very dense plant canopies [13]. Obviously, considerably more investigation is needed before it becomes clear how phytohormones specifically affect, or are affected by, the levels and activity of the different seed phytochromes.

3. Seedling development and growth efficiency

Light is essential for normal seedling development and function. Some of the processes dependent on light include differentiation of leaves and cotyledons, cotyledon expansion, control of stem elongation, chlorophyll production, and the development of the photosynthetically competent chloroplast. CKs have been shown to promote cotyledon expansion, leaf development and chloroplast differentiation [27]. Although these similarities in effect led to the suggestion that R and CKs might operate via the same biochemical mechanisms [102], other observations indicated that CKs did not contribute to the metabolic chain between phytochrome and the photoresponse. For example, although KIN promoted cell enlargement in bean leaf discs [129], R promoted cell division [113]. It was also demonstrated that the effect of KIN in increasing the multiplication rate of *Lemna minor* fronds in darkness was not substitutive for, but synergistic with that of Pfr treatment [123]. Nevertheless, both CKs and Pfr promote the development and affect the efficiency of the photosynthetic apparatus in a similar manner. This has been demonstrated consistently

by exposing dark-grown etiolated tissues to R or CKs. At the cellular level, light exposure induces the transformation of proplastids or etioplasts to photosynthetically competent chloroplasts. This process involves the biosynthesis and assembly of pigments, pigment-binding proteins, reaction centre and electron-transfer complexes, enzymes of CO_2-fixing pathways and other components of the photosynthetic apparatus.

The effect of R pulses on greening can be imitated by CK treatment in that it abolishes the lag phase in chlorophyll production and accelerates its rate [33, 47]. The general effect of CK on chloroplast development has been attributed to probable regulation of certain plastid proteins and thylakoid assembly [1, 45, 108]. Expression of many plastidic genes seems to be induced by both light and CKs [15, 29]. CK activity has also been specifically related to the synthesis of 5-aminolevulinic acid (ALA) [34, 48]. Although BA pretreatment of excised cotyledons of etiolated cucumber stimulated chlorophyll formation during subsequent illumination, the hormone seemed to act independently of R [35]. Since the effects of CK and R sometimes tend to be additive, it was suggested that the causal sequences resulting in chlorophyll accumulation stimulated by either CK or Pfr may be different [50]. One such reaction affected in this way seems to be ALA synthesis [77]. Conversely, it has been suggested that in etiolated cucumber seedlings, BA can completely substitute for phytochrome in down-regulating the accumulation of *PHY-A* mRNA [31]. Intriguingly, this suggests that phytochrome action may be at least partly mediated through increasing the concentration or effectiveness of CKs and/or that CKs may be an important determinant of the capacity for light perception, at least in this system.

Analysis of nuclear genes subject to either induction or repression by CKs indicates that in many, but not all cases, light and CKs operate in the same direction [61]. In particular, CKs increase the accumulation of proteins encoded by light regulated genes involved in photosynthesis, such as *CAB* (encodes the light-harvesting chlorophyll *a/b* binding proteins of PSII) and *RBCS* (encodes the small subunit of RuBisCo) [46, 49, 89, 105, 144]. Similar inductive effects on mRNAs and proteins associated with chloroplast development have been demonstrated by other workers [32, 136, 145]. They also affect the expression of PEPCase [127, 141] and carbonic anhydrase [141]. In etiolated cucumber cotyledons, the level of mRNA transcribed from *psaL*, which encodes a subunit of PSI, was increased by CKs in a manner similar to light treatment [153]. In *Lemna gibba*, KIN regulation of *CAB* and *RBCS* mRNA levels was primarily post-transcriptional [49]. The same workers concluded that R effects on *CAB* and *RBCS* expression are not mediated by CK action, since the inductive effects of the two stimuli were additive [49].

Chory and co-workers [27] demonstrated that micromolar concentrations of CK cause de-etiolation of dark-grown *Arabidopsis* wild-type seedlings in a manner similar to that observed in the *d*e-*et*iolated *det1* and *det2* mutants. This implies that an increase in CK is sufficient to override a light requirement for leaf and chloroplast development in *Arabidopsis*. However, no differences were identified in CK levels in dark-grown *det1* mutants as compared with wild-type plants, although it should be pointed out that the CK level measurements were only averages for the whole plant at one stage of growth. This would take no account of distribution of CKs within specific organs/organelles or at particular 'sensitive' developmental stages. It was suggested that since both of these *det* mutations are recessive, the wild-type genes may act as negative regulators in a CK signal transduction pathway that is coupled to photoreception. Other explanations for the behaviour of both mutants are that DET1 and DET2, directly or indirectly, may inactivate CKs or render them inaccessible through conjugation, or that they negatively regulate CK synthesis or activate CK degradation. On the basis of these and other results, the authors proposed a model in which light and CK act independently or sequentially through common signalling mechanisms involving DET1 and DET2 action to control down-stream light-regulated responses. Based on R and CK treatment of *Lemna*, Flores and Tobin [49] proposed that phytochrome and CK independently alter the pool size of a common intermediate, which then more directly regulates gene expression. Chory et al. [27] suggested that these intermediates may be specified by the wild-type *DET1* and *DET2* genes in *Arabidopsis*. Subsequently, *DET2* was shown to encode a steroid 5α-reductase, which catalyses the first committed step in the synthesis of brassinolide [24]. The recent demonstration that CK downregulates expression of a putative steroid 23-hydroxylase encoded by the *Arabidopsis CPD* (*c*onstitutive *p*hotomorphogenesis and *d*warfism) gene [142], which is apparently involved in one branch of the brassinolide biosynthetic pathway [24], is consistent with the proposed antagonism of CKs and BRs discussed elsewhere in this issue [61].

The close relationship between R and CK effects has been demonstrated in other aspects of seedling

development using *Arabidopsis* mutants. The *amp1-1* (*a*ltered *m*eristem *p*rogram) mutant [22] has 6-fold more Z and DHZ than its wild-type counterpart. When grown in the dark, *amp1* seedlings show morphogenic properties similar to light-grown wild-type plants e.g., they exhibit apical hook opening, limited cotyledon expansion and form etiolated leaves. The adult plant displays severely reduced apical dominance, early flowering and delayed senescence. Explants of *amp1* demonstrated increased potential to regenerate shoot buds in culture. Subsequent analysis indicated that in light-grown *amp1-1*, Z, DHZ and iP levels are approximately 7-, 3- and 2-fold higher respectively than in the wild-type [23]. In dark-grown *amp1-1*, elevations in Z and DHZ levels are approximately 2.5- and 3-fold respectively. Based on these characteristics, it was suggested that *AMP1* might encode a negative regulator of CK biosynthesis, or may be required for the degradation of CKs. One possibility is that the gene might code for, or positively regulate, the activity of CK oxidase [60]. One of the less pleiotropic *c*onstitutively *p*hotomorphogenic mutants, *cop2*, has been shown to be allelic to *amp1* [86]. In view of the generally accepted inhibition of apical dominance by CKs, it is interesting to note that *Arabidopsis hy1* and *hy2* mutants, both of which are impaired in the synthesis of the tetrapyrrole chromophore of phytochrome, have increased apical dominance. The demonstration that *hy2/amp1* double mutants partially restore the *amp1* phenotype suggests that AMP1 action is at least partly independent of the control of a functional phytochrome. Chaudhury et al. [22] suggest that the absence of phytochrome might lead to a reduced level of CKs, or elevated auxin, causing increased apical dominance. For instance, phytochrome mutants of *Nicotiana plumbaginifolia* (*pew*) have only about half the level of Z in their leaves as do wild-types [85], although iP and [9R]iP levels were similar. Alternatively, viewed from the perspective that mutations at the *amp1/cop2* locus partially suppress the phenotype of the *hy2* [22] and *hy1* [70] mutants, the results could implicate a role for CKs in mediating a subset of phytochrome actions. That interpretation of these effects may be far from straightforward is evidenced by the recent demonstration that the *Arabidopsis cri1* (cri*s*t*al*) mutant, like *amp1*, accumulates approximately six times more CK than the wild-type, but displays none of the traits typically associated with CK overproduction which are observed in *amp1* [125].

Heuristic models which attempt to integrate our current appreciation of the involvement of R, CKs, BRs and genetically defined signalling components are presented in Figure 1. Caveats associated with such simplistic representations are that not all lesions in any of the genetically-defined steps have identical effects on photomorphogenesis and that e.g., BRs may primarily participate in pathways controlling cell elongation which are secondarily affected by light. CK and light-response systems were deemed to be independent and additive in relation to the inhibition of hypocotyl elongation in *Arabidopsis*; either CK or light could saturate this morphogenic response [140]. In six *hy* mutants, which apparently have normal CK responses, the effects of CK on the inhibition of hypocotyl elongation are largely mediated by ethylene; blocking the ethylene response pathway by using an *e*thylene-*in*sensitive mutant (*ckr1/ein2*) had no effect on the light inhibition of hypocotyl elongation. These results suggest that CKs do not directly mediate the action of light on hypocotyl elongation. The authors concluded that two signalling pathways are involved in the hypocotyl elongation of wild-type and light-insensitive *Arabidopsis* mutants. Nonetheless, they noted that the *phyB-1* mutant exhibits a modified response to CK, suggesting a link which might be indirect, between CKs and phyB signal transduction, at least as regards hypocotyl elongation. It would seem that a functional GA system is also necessary for full expression of stem elongation [109], suggesting that light inhibition of hypocotyl elongation could be more related to GA deficiency rather than CK expression. R treatment causes hypocotyl disorientation of *Arabidopsis* seedlings, by interfering with the gravity-sensing system. CK can cancel this effect, also probably through stimulating ethylene production, as demonstrated in experiments with an ethylene insensitive mutant (*ein 2-1*) [53].

The above results demonstrate that the implication of specific hormones in physiological processes controlled by light will not easily be clarified because of complex interactions occurring between the hormones themselves. Equally, it seems that interactions between different phytochromes may be a further complication. It is suggested that the antagonistic and complementary actions of phyA and phyB are necessary for the optimal regulation of seedling growth and development after emergence from the soil [134].

Current information relating to the molecular basis of CK action, particularly the involvement of CK-binding proteins, intracellular Ca^{2+}, protein phosphorylation and DNA/protein methylation is reviewed elsewhere in this issue [61]. Microinjection experiments which have demonstrated that Ca^{2+}-activated

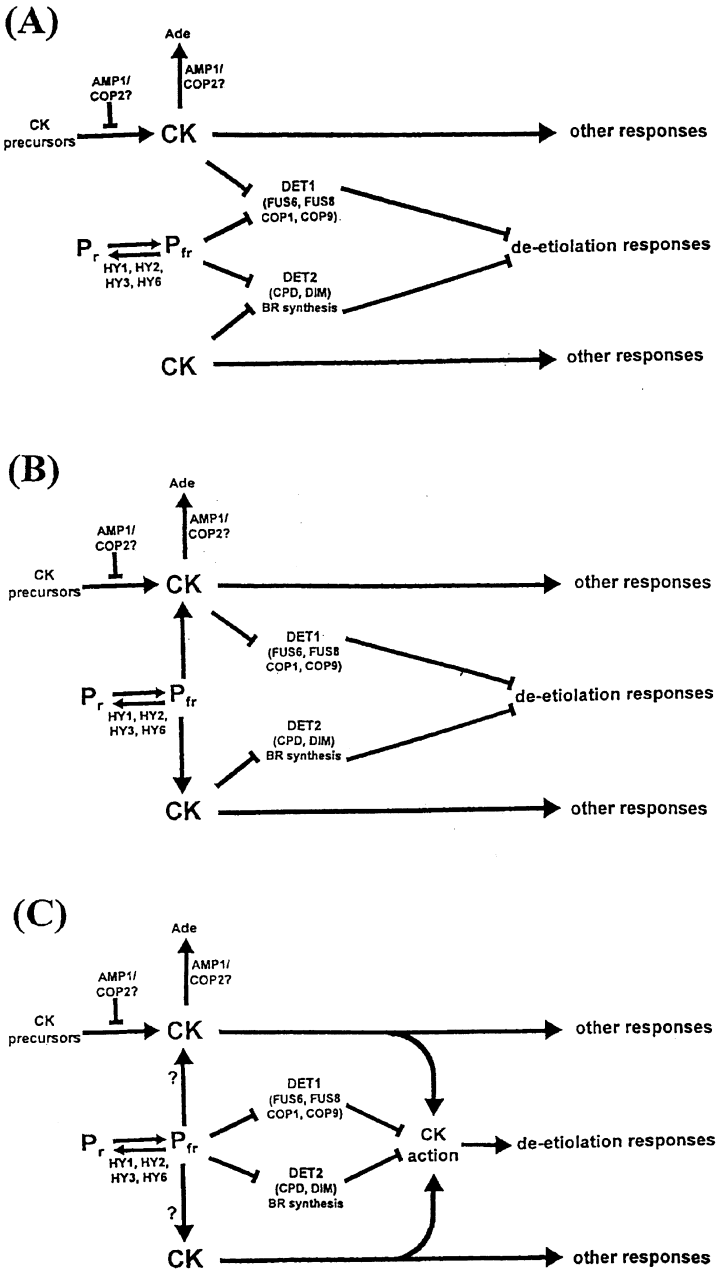

Figure 1. Models for the effects of light, CKs, BRs and *AMP1*/*COP2*, *DET1* and *DET2* genes on light-regulated processes in *Arabidopsis*. **(A)** DET1 and BR synthesis are repressed independently by either light or CKs. **(B)** Activation of CK action, possibly *via* stimulation of CK accumulation by R [115, 157, 158], downregulates DET1 action and/or BR synthesis. **(C)** Inactivation of DET1 activity or suppression of BR synthesis by light increases the sensitivity to CKs of a phototransduction pathway(s) that involves CK action. None of the models are mutually exclusive. Photomorphogenic responses controlled by light and CK involve gene expression, chloroplast differentiation, leaf expansion and the inhibition of hypocotyl elongation. DET1 and DET2 are believed to define BR-independent and -dependent pathways respectively [26]. The DET1 branch also involves the action of COP1 and a protein complex which includes COP9, FUS6 and FUS8 [21, 26, 137], all of which are apparently localised in the nucleus. Both CPD and DIM have been proposed to participate in the BR-dependent DET2 pathway [24]. AMP1/COP2 [22, 23, 86] may repress cytokinin synthesis or remobilisation from storage forms and/or activate CK degradation. Elevated CK levels in *amp1* are associated with phenotypes (e.g., early flowering, altered phyllotaxy and floral morphology, normal stature, normal anthocyanin levels) different to those associated with mutations in *DET1* or *DET2* [22, 23]. Although DET3 and HY5 act downstream of DET1 and mediate light-induced inhibition of hypocotyl elongation and leaf expansion [25], their relation to CK-mediated photomorphogenesis is not well defined. Ade, adenine; $\rightarrow$, positive action; $\dashv$, negative action; ?, optional. After [23, 27, 142].

CaM participates in the signal transduction chain linking light-perception to the induction of some, but not all, phytochrome-regulated genes [14] are discussed in Section 4 of this review. In excised cucumber cotyledons, external Ca^{2+} is required for greening and the enhancement of greening by pre-exposure to either light or CK [121]. Nifedipine, a substituted dihydropyridine, prevented an enhancement of chlorophyll accumulation by treatment with BA but not by R, while another Ca^{2+}-channel blocker, Nd^{3+} (neodymium) prevented light-induced enhancement of chlorophyll accumulation, but had no effect on CK enhancement of this process. The differential inhibition of greening by light and CK using these two Ca^{2+}-channel blockers suggest that R- and CK-induced chlorophyll accumulation in etiolated cucumber cotyledons are mediated by different classes of Ca^{2+}-channels and that only the class modulated by CK is effectively blocked by nifedipine [121]. The fact that certain CaM antagonists can block the enhancing effect of CK on chlorophyll accumulation [162] strengthens the view that a Ca^{2+}/CaM system is involved in photomorphogenic effects brought about by CK and R. Recent evidence, also based on the use of selective inhibitors of Ca^{2+} channels, suggests that CK- and R-induced membrane depolarisation and caulonemal side branch initial formation in the moss *Physcomitrella patens* are mediated by different classes of Ca^{2+} channels in the plasma membrane [43].

4. Pigment synthesis

A further response of plants to CK treatment is the accumulation of pigments such as anthocyanins and betacyanins. The synthesis of betacyanins in *Amaranthus* spp. seedlings is promoted by CKs and R [3, 9, 81, 111, 112] and inhibited by GA [80]. It was also reported that ABA inhibited light-promoted betacyanin synthesis in *A. tricolor* [139]. Using the *A. caudatus* bioassay, it was demonstrated that the effects of ABA and GA on betacyanin synthesis tended to be additive and that ABA inhibited the promotive effect of Z [8]. The promotive effects of light and CKs on betacyanin synthesis appeared to be additive or slightly synergistic [82, 55] when light treatments were 4 h or less, but when 24 h illuminations were supplied KIN had no additive effect. Thus, when the response to light is saturated, there is no further effect of CK, suggesting that CK and light may exert their effects at the same site or *via* the same pathway.

Anthocyanins are pigmented flavonoids that are responsible for most of the red, pink, purple and blue colours found in plants. That genes in the anthocyanin synthesis pathway are regulated by light is well documented [154]. BA also increases anthocyanin accumulation, but only in the presence of light [37], a phenomenon similar to that occurring in relation to seed dormancy-break. Anthocyanin synthesis is controlled in part by the key enzymes phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS), both of which are regulated by light at the transcriptional level. In *Arabidopsis*, low temperature exposure increased the steady state levels of *PAL* and *CHS* mRNAs only in the light [90]. *Arabidopsis* plants treated with CKs accumulate anthocyanin pigments, with levels of transcripts encoding four key enzymes in the biosynthetic pathway, *PAL1*, *CHS*, chalcone isomerase (*CHI*) and dihydroflavonol reductase (*DFR*) increasing coordinately in response to BA [37]. Although *CHS* and *DFR* seemed to be controlled by BA at the transcriptional level, *PAL1* and *CHI* are controlled post-transcriptionally. The fluctuations of these transcripts during a 24 h period and the possible implication of CK involvement in regulating circadian rhythmicity of gene expression are discussed in Section 5. Assuming that the genes involved in anthocyanin biosynthesis in *Arabidopsis* are coordinately regulated by a common induction pathway, this study suggested that CK regulation of anthocyanin gene expression in *Arabidopsis* may occur via a different signal transduction pathway from light, since only *CHS*, and not *PAL1*, appears to be transcriptionally activated by CK. In view of the links between CK action and de-etiolation discussed above, it is interesting to note that elevated levels of transcripts encoding *CHS* and *PAL1* are observed in photomorphogenic mutants *det1-1* and *cop1-1* [95]. *CHS* is also overexpressed in the *Arabidopsis cop9-1* mutant [95] and a moderate increase in *CHS* transcript was observed in dark grown *amp1-1* relative to the wild-type [23]. Anthocyanin levels are not altered in *amp1* mutants [23]. Interestingly however, upon transfer of light-grown *amp1* plants to the dark, the expression of *CHS*, *RBCS* and *CAB* genes was repressed weakly compared with larger decreases in wild-type *Arabidopsis* [23], thus suggesting a possible link between CK levels and dark-adaptation processes. In contrast, expression of light-regulated genes remains high after transfer of *det2*, *cop1* and *cop9* to darkness, while *det1* and *cop4* mutants exhibit wild-type responses to dark adaptation.

BA weakly induces expression of genes involved in anthocyanin biosynthesis in petunia corolla tissue [103]. Although simultaneous treatment with BA and GA induced the activity of four key enzymes additively, addition of the inhibitory hormones ABA and NAA seemed to affect the GA- and BA-induced pathways differently. Both compounds inhibited the expression of GA-induced CHS and anthocyanin synthase (As). They also selectively inhibited the expression of BA-induced DFR and As but not that of CHS and CHI. This may be explained by differential control of two clusters of anthocyanin pathway genes, the occurrence of which is supported by genetic data.

It was demonstrated that R and CK promotion of anthocyanin synthesis in red cabbage was inhibited by membrane stabilizing compounds [63]. This suggested that both light and CKs induce anthocyanin synthesis by some mechanism involving the alteration of membrane permeability. As in the case of seed dormancy, the *Amaranthus* betacyanin synthetic system is stimulated by fusicoccin and influenced by Ca^{2+} [40]. Investigations using CaM-binding compounds also indicated that the Ca^{2+}-dependent regulator protein CaM may be involved in pigment synthesis [41, 42]. These results seem compatible with a mechanism of control involving selective effects on ion transport common to CK, R and fusicoccin action together with effects on membrane function and integrity.

Seedlings of the *aurea* mutant of tomato, which is deficient in phytochrome chromophore biosynthesis, fail to develop normal chloroplasts or to synthesise anthocyanins in response to light. Microinjection experiments have demonstrated that wild-type photoresponsiveness, including the production of pink anthocyanin pigments and mature chloroplasts as well as activation of a *GUS* reporter gene fused to a wheat *CAB* promoter (*CAB::GUS*), is restored upon introduction of purified oat phyA into the mutant tomato cells [104]. The role of phyA in induction of these processes could be fulfilled by G-protein agonists, whereas blocking the action of heterotrimeric G-proteins prevented activation of the reporter gene by phyA. Microinjection of Ca^{2+} or activated CaM into *aurea* cells, either of which could stimulate *CAB::GUS* expression and trigger the production of immature chloroplasts lacking PSI and cytochrome b_6f, had no effect on anthocyanin synthesis [104]. Using the same methodology, it was subsequently shown that cGMP activates a *CHS::GUS* construct and thus mediates anthocyanin synthesis [14]. cGMP did not induce expression of a *CAB::GUS* reporter gene. Development of complete chloroplasts occurred only after co-injection of Ca^{2+} and cGMP. The promoter of the ferredoxin $NADP^+$ oxidoreductase (*FNR*) gene was fused to GUS (*FNR::GUS*) as a marker for transcription encoding components of PSI. Whereas microinjection of Ca^{2+} or cGMP alone was insufficient to stimulate *FNR::GUS* expression, simultaneous injection of both Ca^{2+} and cGMP was necessary and sufficient for activation of the marker gene.

Taken collectively, these data suggest that phyA signalling involves at least three pathways, one of which involves Ca^{2+} and regulates *CAB* and other components of PSII, another involving the cGMP-dependent expression of *CHS*, and the third a combination of both second messengers, which is necessary for the production of mature chloroplasts and regulates the expression of genes encoding components of PSI. All three branches are dependent on the activation of one or more heterotrimeric G-protein(s). Since both Ca^{2+} and cGMP carry only minimal intrinsic informational specificity, one of the most significant implications of these studies is the suggestion that hypocotyl cells are preprogrammed to respond fully to both signalling intermediates. Furthermore, light does not appear to be required for the execution of any of the phytochrome phototransduction processes downstream of G-protein action [104].

Our current understanding of the photocontrol of *CHS* expression, including the involvement of a genistein-sensitive (tyrosine) protein kinase has recently been reviewed [126]. Besides phyA, blue/UV photoreceptors also strongly control *CHS* expression in some developmental stages. The UV pathway also includes several trimeric G-proteins, but in contrast to the phyA-dependent pathway, Ca^{2+}/CaM and not cGMP appear to participate in the transduction cascade [126]. Exactly how CKs may be involved in such a scheme remains to be determined. Genes such as *CHS* apparently integrate several endogenous (e.g., CKs) and environmental (e.g., light, stress factors) signals, some of which act in concert. The likely existence of cell- and tissue-type specific signalling intermediates, themselves possibly under developmental regulation, may introduce further complexity to the regulatory networks controlling the expression of such genes. It remains to be established whether overlapping or separate pathways are used to transduce these signals and whether different members of multigene families specifying components of the transduction pathway indicated above are used for each signal.

5. Photoperiodism and flowering

Plants regulate light-dependent processes not only by continuously maintaining the capacity to respond to the perception of this vital cue, but also by predicting the onset and duration of the light period using an endogenous timing mechanism. Under natural conditions, many biochemical and physiological processes oscillate with 24 h periodicity and much has been written concerning the effects of photoperiod on plant morphology and function, particularly with respect to flowering. In order to measure the duration of light and dark, plants require both a time-measuring system and a photoreceptor. The hypothesis by Bünning [17] that time measurement involves an endogenous circadian rhythm, with some of the cycle passing in darkness for induction to occur, is now generally accepted [69]. This rhythm is reflected by changes in morphology and the flowering response to increasing durations of darkness, or to light interruptions during an otherwise inductive dark period. That an internal circadian clock is synchronised daily to natural photoperiods is further evidenced by the demonstration that biological rhythms generally persist under conditions of constant light and temperature with a periodicity close, but not equal, to 24 h [96]. To time the duration of darkness, endogenous rhythms must be phased from the end of the light period. Evidence that phytochrome is the receptor involved in the control of phase is still not definitive. The interactions between phytochrome and rhythmicity have been reviewed [92]. It is clear that the effect of a light break on development in short-day plants (SDP) involves a direct action of phytochrome since some elements of the process are R/FR reversible in a number of species [159]. The interaction of phytochrome and rhythms in long-day plants (LDP) is less well understood.

Recent evidence is consistent with the view that the rapid regulation of light-induced genes and their control over a 24 h time scale are not independent [99, 101]. Regarding CKs, it is striking that many CK-controlled genes display rhythmic patterns of expression [61]. Intriguingly, a circadian rhythm exists for endogenous CK concentration in carrot [138]. Thus, events downstream of CK perception might be integrated with pathways responsible for biological timekeeping or regulation of CK action might be an important output from the endogenous timing mechanism. Since the first report of an increase in CK levels, as measured by bioassay, in *Begonia* leaves exposed to SD [67], several researchers have investigated the possible involvement of CK action in the photoperiodic regulation of the floral transition. Since it had been shown sometime earlier [56, 57] that CK application could induce flowering in some SDPs under non-inductive conditions, it was suggested that at least part of the floral stimulus induced by photoperiodic change may be under CK control. In an intriguing experiment [12], it was shown that lettuce seeds cv. Grand Rapids implanted into the petioles of induced *Xanthium strumarium* plants germinated better than those in the non-induced plants, both in the light and dark. This suggested that a hormonal component, possibly GAs and/or CKs, could be involved in the induction signal. Subsequently, higher concentrations of CKs were reported to be present in the phloem sap of photoperiodically-induced *Xanthium* plants than in those which were non-induced [110]. Further studies on this species [158] indicated that within 10 d of the commencement of SD treatments, there was a change in the overall distribution of CKs in the plant, with induced plants having relatively large quantities in their meristematic regions and very little in the mature leaves. CKs present in the meristematic regions of the plant were mainly in the form of free bases and ribosides, whereas the mature leaves contained mainly CK ribotides. Night break treatments during a 16 h dark period also resulted in large effects on CK distribution in the plants, indicating a true photoperiodic effect on CK metabolism and/or distribution.

Subsequent studies with *Xanthium* [65] indicated that the CK content of leaves and buds of intact plants were reduced significantly in response to one long dark period. Since no substantial differences in the rate or pattern of Z metabolism in detached *Xanthium* leaves or buds due to photoperiod were observed [66], it was concluded that photoperiodic effects on CKs in plants were probably due mainly to differences in transport rather than metabolism. However, in poplar (LDP), CK levels in both attached and detached mature leaves increased transiently after short exposures to R, the major CK affected being 6-(2-hydroxybenzyl)aminopurine riboside [68].

In *Begonia*, CK levels were higher in conditions favourable to flowering i.e., low temperature or LD [59]. Further, a correlation between exudation of CKs from leaves and flower induction was demonstrated in *Perilla frutescens* [54], with more [9R]iP and [9R]Z exuded from leaves exposed to SD than those kept in LD or given night-break treatments. Increases in CK levels in both xylem and phloem exudates in the LDP *Sinapis alba* after an inductive treatment of 16 h

light have been demonstrated [88]. Additional attempts to relate photoperiodic induction of flowering to CK changes were approached using the related species *Chenopodium rubrum* (SDP) and *C. murale* (LDP), which have different photoperiodic requirements [93]. Since similar changes in a spectrum of CKs, including Z, [9R]Z, iP and [9R]iP occurred under the same light regime, it was concluded that there is no correlation between CK levels in leaves, stems and roots and photoperiodic induction of flowering. However, subsequent experiments indicated that changes in CK transport following photoperiodic treatment in *C. rubrum* plants, possibly unrelated to the flowering stimulus, are definitely under phytochrome control [94]. In these experiments, a 15 min R light break almost completely prevented the promotive effect of darkness on CK concentrations and its effect was reversed by FR irradiation. On comparing the composition of individual exudates, it was concluded that CKs arriving at the apical region were derived mostly from leaves, with varying contributions from the roots.

Bernier [5] suggests that the photoperiodically-induced floral stimulus is multi-factorial, with sucrose being a major component. In *Sinapis alba*, both iP and the polyamine putrescine also seem to be involved in the signal [64, 87, 88]. In addition to action at the shoot apical meristem, components of the floral stimulus appear to be acting elsewhere in the plant. For example, the export of CKs in the xylem sap of *S. alba* seems to be influenced by the action of sucrose in to the root system [6]. CK treatment causes changes in the cell division process in the apical meristem, but additional effects are observed when both CK and sucrose are applied [5, 6]. Photoperiodic induction effects, particularly in SDPs, are associated with the presence of functional phyB [133]. Photoperiodic perception involves both HIR and R/FR reversible responses. Earlier flowering of *amp1* mutants of *Arabidopsis* is consistent with the possibility that CK is involved in flower induction, acting together with GA in floral transition [22]. The authors proposed a model of flowering in which CKs and GAs control two independent pathways.

Many LDPs can be induced to flower early under low R/FR ratio light conditions. This appears to be a separate phenomenon from the photoperiodic induction of flowering and more related to the shade avoidance strategy adopted by plants. Although phyB is involved in the low R/FR response, there is evidence that a photoreceptor other than phyB may also be important [161]. Indeed, the response of a *phyB Arabidopsis* mutant to low R/FR is similar to that of the wild-type [122]. Genetic studies involving a range of *Arabidopsis* mutants have confirmed this involvement of more than one phytochrome in the low R/FR response. For example, the *hy3* mutant, which lacks immunologically detectable phyB has been used in late-flowering background crosses to accelerate flowering in response to low R/FR [58]. Overexpression of phyA in transgenic *Arabidopsis* accelerates flowering under SD and under continuous white light, unless supplemented by FR [2]. It seems that in light-grown seedlings, phyA modulates the extent of response to reductions in R/FR ratio perceived by phyA, perceives daylength extensions and night interruptions affecting flowering, and perceives light treatments resetting endogenous rhythms [18].

At the molecular level, a link between CK action and entrainment of the endogenous timing mechanism is not straightforward. Presently, we cannot dismiss the possibility that the relationships between CK and circadian variations in gene expression may simply be correlative, especially when these genes are also responsive to light. Genes either induced or repressed by CK display circadian oscillations in their levels of abundance, with maximal expression during the light period [61], while transcripts encoding enzymes in anthocyanin biosynthesis displaying induction by CK at both the transcriptional and post-transcriptional levels were highest during the dark period [37]. Exogenous CK dampened these diurnal fluctuations [37]. In contrast, CK increased the amplitude of rhythmic oscillations in levels of transcript encoding an early nodulin gene [132]. Although CK apparently mediates accumulation of *CAB* transcripts by post-transcriptional regulation [49], both transcriptional and post-transcriptional regulation by the endogenous pacemaker has been reported for *CAB* genes in *Arabidopsis* [96]. In *Arabidopsis*, CK-mediated increases in *CAB* levels must be at least partly due to increased transcription rates, since a promoter was adequate to increase activities of marker gene constructs [27]. Accumulation of transcript encoding nitrate reductase (NR) by CK is transcriptionally regulated [61], although the NIA2 isoform of NR from *Arabidopsis* is subject to primarily post-transcriptional regulation by the circadian clock [96].

In tobacco and *Arabidopsis*, circadian oscillations in levels of Ca^{2+} can be phase-shifted by light-dark signals [72]. The involvement of shifts in protein phosphorylation status in facilitating circadian rhythmicity is also evident since phosphoprotein phosphatase inhibitors alter circadian properties in the dinoflagel-

late *Gonyaulax polyedra* [30]. These ubiquitous signalling mechanisms could thus represent points of convergence of CK action and pathways which enable plants to anticipate changes in the light environment. It would be interesting to investigate whether mutations that shorten the periodicity of *CAB* gene expression [100] also affect the periodicity of other CK-regulated genes. Both *det1* and *det2* mutants, which have an altered response to CK [27], exhibit shorter circadian periods in constant darkness [101]. However, although the *amp1/cop2* mutation causes an elevation of endogenous CKs in base, riboside and nucleotide forms as well as increased levels of transcripts encoding light-regulated genes, diurnal control of *CAB* gene expression was unaffected in this mutant [23]. It is regrettable that the effects of transgene-mediated CK overproduction in *Arabidopsis* have not been investigated as extensively as in tobacco, considering the eminence of this species in plant molecular genetics. However, in the only reported study in which this was attempted, it is interesting to note that thermal induction of *ipt* expression in plants grown in LD (16 h photoperiod) had no effect on plant morphology, whereas heat treatment under SD conditions (8 h) reduced xylem proliferation [98].

6. Light, CKs and nutrient status

The well-documented involvement of CKs in regulating light-responsive genes involved in photosynthesis, sucrose transport and nitrate assimilation [61] implicates their participation in the control of nutrient metabolism. Evidence supporting this notion has arisen from studies involving CK-related mutants [36]. Recent studies demonstrating the potent signalling roles of sugars [71, 83] and nitrate [128] introduce the question of how light and CK action may interact with or regulate nutrient signals that control growth and development. Characterisation of recently identified *Arabidopsis* mutants [39, 91] has implicated an important role for light in modulating plant responses to both carbohydrate and nitrogen sources. Expression of several CK-responsive genes, including *CAB*, *RBCS*, *NR* and *CHS* are disrupted in sucrose-*un*coupled (*sun*) mutants [39] and *cr88*, which defines a new *HY* locus [91].

Besides acting as substrates for growth, sugars are important signals capable of controlling plant gene expression and development. Many developmental processes under CK- and/or phytochrome control, including germination, cotyledon greening, hypocotyl elongation, primary root growth, true leaf development, control of apical dominance, the transition to flowering and the onset of senescence, are influenced by photosynthetic end-products [71, 83]. Sugars not only feedback regulate photosynthesis at the level of electron transport and enzyme activity, but also through repression of nuclear genes encoding photosynthetic proteins including CAB and RBCS [71]. It has been suggested that sugar signals overlap with CKs and other hormones in regulating the expression of several genes [83]. A combination of CK and sucrose decreased levels of an *Arabidopsis* transcript encoding a cytochrome P450 apparently involved in BR biosynthesis, particularly in the light [142], and a synergistic effect noted between sucrose and low levels of CKs was suggested for induction of accumulation of transcript encoding a D-type cyclin [135].

Nitrate application has been associated with developmental alterations such as increased shoot/root ratios [128], and delayed flowering and senescence [6]. Coupling of the nitrate assimilation genes to those associated with the regulation of photosynthesis provides a mechanism whereby plants may coordinate both processes to ensure effective partitioning of energy. Conceivably, mechanisms must also exist to integrate nutrient status with other aspects of growth and development. Consistent with the interactive effects of nitrate and sugars at the level of their absolute concentrations, many of the genes regulated by signals deriving from nitrate *per se* (and not nitrogen metabolites) are also affected by sugars [128]. Although induction of NR by light can be replaced by exogenous sugars, the effects of low light suggest the involvement of a phytochrome-mediated component. The observation that nitrate induces accumulation of transcript encoding PEPCase and enhanced PEPCase activity [128] may have some bearing on the demonstration that CK is required to induce the N-dependent accumulation of PEPCase and carbonic anhydrase in detached maize leaves [141].

The role of CKs in mediating plant responses to mineral nutrient deprivation and other stresses is covered elsewhere in this issue [62]. Therefore, although some evidence indicates that phytochrome and CKs act independently, it seems equally possible that light, CKs and nutrient assessment pathways could participate in a common circuit of the signalling network that links the perception of vital environmental cues with growth and development. Accordingly, CK-induced expression of a light- and nutrient deprivation-regulated wheat

gene, WPK4, which encodes a protein kinase, was demonstrated by its inactivity in the presence of a CK antagonist [124]. Dissecting out the apparent overlaps between light, CKs and nutrient signals is likely to be an important challenge in elucidating the role of CKs in enabling plants to respond to their environment in an integrated manner.

7. Conclusions

Prior to the current extensive genetic studies being carried out, particularly with *Arabidopsis* mutants, there was considerable indication that phytochrome and CK action were closely linked. Both physiological and genetic evidence also implicates complex interactions between CKs and other plant hormones, the actions of which may also be affected by light perception. For example, the dwarf phenotype of tobacco plants overexpressing oat phyA is related to a reduction in endogenous GA levels since this can be overcome by foliar application of GA_3 [74]. However, the inability of GA_3 to suppress traits such as increased pigmentation, partial release of apical dominance and delayed leaf senescence implicates a disruption in CK action, although preliminary data indicated no change in CK levels in these plants [74].

Presently, a rapidly accumulating body of information indicates that a wide spectrum of hormones, probably acting in concert together with light, sugars and amino acids, are involved in complex regulatory networks mediating developmental transitions as well as modulating aspects of vegetative growth such as the expression of photosynthetic genes and those involved in nitrogen assimilation. None of these factors act in isolation and the emerging complexity of gene regulation suggests that the signalling capacities of light and nutrients may largely be determined by the prevailing hormonal balance. Certainly, the exact roles of the different classes of hormones in photocontrol are perplexing, owing largely to considerable difficulty in unambiguous interpretation of much of the data currently available from research involving mutants. Genetic models of phototransduction pathways in *Arabidopsis*, such as the one shown in Figure 1, do not address the actual mechanisms involved in light signalling. CKs may be involved in or affected by DET and COP functions as part of the photoresponse, depending on whether they act before or after *COP* and *DET* products. While preliminary biochemical evidence supports the involvement of Ca^{2+}, CaM, cGMP, G-proteins and phosphorylation in phytochrome signalling, there is no indication of how these ubiquitous signalling components interact in the context of macromolecular intermediates implicated in eliciting the effects of either R or CKs.

Regarding CKs, on the positive side, recent studies indicating that high CK levels can induce photomorphogenic phenotypes in dark-grown wild-type plants at both the levels of morphology and gene expression, together with the observation that some phenotypes (e.g., slower senescence and reduced apical dominance) of light-grown *det* and *cop* mutants resemble wild-types that have been supplied with CKs or transgenic plants that overproduce CK, provide convincing evidence of a close link between the actions of phytochrome and CKs. *Arabidopsis det1* and *det2* mutants also demonstrate differences in callus culture growth and the capacity to regenerate buds and roots [27]. Nonetheless, these results are offset by indications, albeit with some reservations [61], that light-grown *det1* and *det2* mutants do not have increased CK levels [27]. Some, but not all CK-overproducing mutants, exhibit impaired dark-adaptation responses. The application of transgenic *ipt*-expressing plants that overproduce CKs to examination of phytochrome mediated events has not yet been exploited to its full capacity.

It is clear from the information presented in this review that the question whether light and CKs act independently, or whether their respective signalling pathways employ a common regulatory network of interactions to orchestrate appropriate biochemical and physiological responses, remains open. Another complication in unravelling the hormone/photocontrol mechanism is the evidence that hormones do not act independently of each other, as exemplified by the apparent interaction between GAs, CKs and ABA in the control of seed dormancy release [148]. Similar interactions can be demonstrated with *Arabidopsis* mutants. For example, the *fus6* phenotype is partly reverted by exogenous CK and by the insensitivity to ABA caused by the *abi3-3* mutation [19]. An inverse relationship between light and ABA concentrations demonstrated in *Arabidopsis* and *Lemna gibba* [160] may also have some bearing on phytochrome/CK interactions, especially considering the well documented antagonism between these hormones.

There can be little doubt that progress in elucidating these effects must depend on a more complete understanding of the molecular mechanisms of both phytochrome action and early events in CK signal transmission. The observation that all known *Ara-*

bidopsis hy mutations, with the exception of mutations in *HY5*, render photoreceptors non-functional [25], suggest that few positively acting components downstream of the photoreceptors are specific for the light response. The advent of biochemical complementation techniques [14, 104] has provided convincing evidence that G-protein activation independent of R perception can elicit all of the R responses tested thus far. This suggests that investigation of the involvement of heterotrimeric G-protein action in CK signal transmission is the most obvious immediate priority for physiologists interested in elucidating CK/phytochrome interactions at the molecular level. Components that couple phytochrome photoconversion to G-protein regulated pathways have yet to be identified. These may also represent a nexus between the two signals and a variety of downstream regulatory cascades controlling aspects of growth and development subject to control by both stimuli. Recently, on the basis of the activity of a human homolog of COP11, it was speculated that FUS6/COP11 may repress heterotrimeric G-protein-potentiated signalling [21].

In the future, the availability of mutants deficient in CK perception may facilitate the extension of microinjection techniques to investigation of the early events in CK action. In this regard, recent evidence implicating a pivotal role for a two-component signal transduction pathway in certain responses of *Arabidopsis* to CK [75] is encouraging. Likely events downstream of CK perception by CKI1 are discussed elsewhere in this issue [61]. This may also have some bearing on recent evidence that cyanobacterial phytochrome-regulated photoreceptors show similarities to plant phytochrome C-terminal sequences and the histidine kinase domain of two-component sensors [78, 117]. Reports which have implicated the involvement of phosphorylation cascades in phytochrome responses have been reviewed [26, 117, 118]. Intriguingly, recent mutational analysis of phyA and phyB indicated that a common region on the C-terminal half of both photoreceptors is important for signal transmission to downstream components [117]. In view of the well-documented capacity of two-component type systems to integrate disparate signals [61], it is tempting to speculate that if phytochromes are photoregulated histidine kinases, the putative membrane-bound CK-receptor CKI1 [75] and cytoplasmic phytochromes may interact with a common soluble downstream response regulator, thereby enabling CK and R to feed into a single pathway capable of inducing a common subset of genes. Nonetheless, the recently demonstrated widespread distribution of histidine kinase/response regulator systems in both prokaryotes and eukaryotes suggests the early evolution of the two-component signal transduction mechanism. Whether phytochromes found in higher plants have evolved mechanisms that do not involve a histidine-aspartate phosphorelay is presently equivocal [78, 117].

Furthermore, while the simplicity of such a mechanism is attractive, in much the same way that the existence of multiple differentially-regulated phytochromes, each apparently performing both discrete and overlapping roles, may in large part account for the heterogeneity of effects elicited by the phytochrome system, so there may be multiple sites of CK perception and thus more than a single event to which all CK effects can be traced [61].

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