Regulation of gibberellin biosynthesis by light

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Phytochromes regulate transcript levels of gibberellin biosynthesis enzymes, GA 20-oxidases and/or GA 3β-hydroxylases, in germinating lettuce and *Arabidopsis* seeds and in de-etiolating pea seedlings. Feedback regulation of GA biosynthesis by active GA is well established, but other mechanisms for regulation of these biosynthetic genes also exist, as this feedback does not operate on a GA 3β-hydroxylase gene of *Arabidopsis* during seed germination.

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Abbreviations

FR	far-red light
GA	gibberellin
LD	long day
phy	phytochrome
R	red light
SD	short day

Introduction

Although there are extensive studies to implicate gibberellin (GA) biosynthesis and metabolism in the phytochrome-mediated control of different physiological processes (e.g. seed germination and seedling growth), the supporting evidence is sparse. Recently, many genes encoding GA biosynthesis enzymes have been cloned (Figure 1) [1,2,3•,4,5••] and this has made it possible to study phytochrome regulation of GA biosynthesis at the molecular level. Research on lettuce seed germination led to the discovery of phytochrome almost half a century ago, and it was later suggested that phytochrome affects germination through GAs [6]. The regulation of GA biosynthesis appears to be more complicated in seedling growth than in seed germination [1]. Transfer of etiolated pea seedlings to light changes the expression of GA 20-oxidase genes. Tuber formation of potato is also regulated by phytochrome and a role of GAs in that process has been suggested [7•]. This review highlights recent progress in understanding the regulation of GA biosynthesis by phytochromes in selected developmental processes.

Phytochrome regulation of GA biosynthesis during seed germination

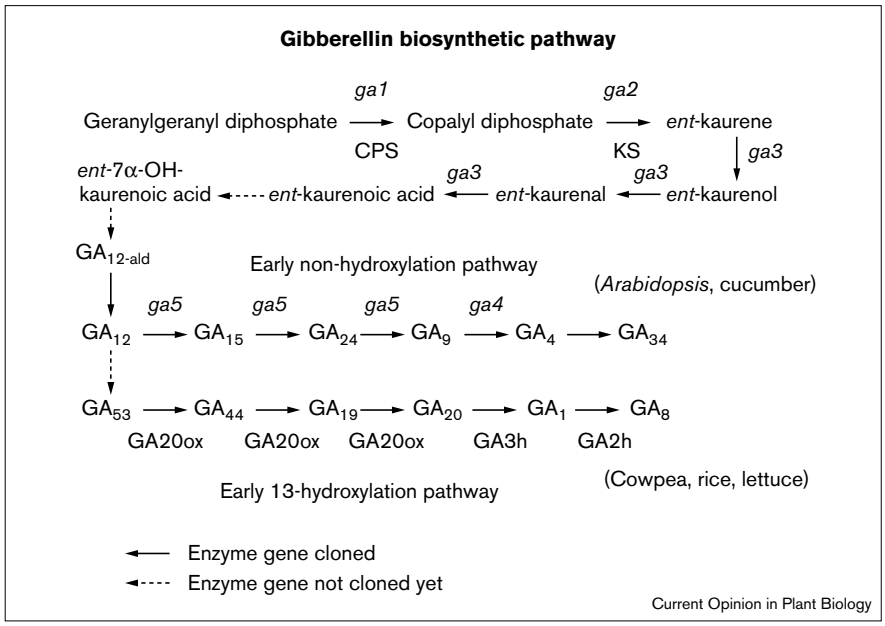
Seed germination is complex and is regulated by many factors such as nutrients, temperature, water and light [8]. Despite extensive studies on the roles of phytochrome in light-stimulated seed germination [9,10,11•,12], the molecular mechanisms of hormonal involvement in this process are largely unknown. The cloning of GA biosynthetic enzyme genes in lettuce and in *Arabidopsis* has enabled the study of these mechanisms [13••,14••].

The endogenous content of GA_1 — the main biologically active GA in lettuce seeds — increases after red light (R) treatment [15]. GA_1 is synthesized from GA_{53} by two different 2-oxoglutarate dependent enzymes, GA 20-oxidase and GA 3β-hydroxylase, acting successively as shown in Figure 1. Lettuce seeds contain high levels of GA_{20} (about one hundred times higher than of GA_1) [15]. Two GA 20-oxidase genes (*Ls20ox1* and *Ls20ox2*) and one GA 3β-hydroxylase (*Ls3h1*) are expressed in germinating seeds (Figure 2a). The expression of *Ls3h1* is induced by red light treatment and this effect is canceled by far-red light (FR) treatment. Expression of *Ls20ox1* and *Ls20ox2* is induced by imbibition alone in the dark. The level of *Ls20ox2* mRNA decreases with red light treatment, whereas that of *Ls20ox1* is unaffected by light. These results suggest that red light promotes GA_1 synthesis by inducing *Ls3h1* expression via phytochrome action [13••]. It is well known that GA 20-oxidation/accumulation of GA 20-oxidase mRNA is regulated by negative feedback of active GAs [1]. Therefore, the down-regulation of *Ls20ox2* expression could be the result of the increased GA_1 content in germinating seeds. Interestingly, although the exogenous application of high levels of GA_1 decreases the expression of *Ls3h1*, this gene was not affected by the increase of endogenous GA_1. Thus, there may be some mechanism to suppress the feedback regulation of *Ls3h1* during seed germination of lettuce.

Arabidopsis ga4-1 is a GA deficient semi-dwarf mutant [16]. The *GA4* gene was cloned by T-DNA tagging [1,4] and it was shown to encode a GA 3β-hydroxylase [17]. Severe alleles of the GA-deficient mutants *ga1* [1,4], *ga2* [18] and *ga3* [3•,19•] fail to germinate without exogenous application of GAs [16], whereas even the putative null allele, *ga4-2*, can germinate without GAs, suggesting the presence of another GA 3β-hydroxylase in germinating seeds. Recently, a *GA4* homolog (*GA4H*) was isolated and shown to encode a GA 3β-hydroxylase [14••]. The *GA4H* gene was found to be predominantly expressed during seed germination. Both *GA4* and *GA4H* genes in imbibed seeds are induced by red light treatment (Figure 2b). At least five loci in *Arabidopsis* [20] encode phytochromes. Among these, *PHYB* encodes phyB, which plays a major role in germinating seeds shortly after the start of imbibition [9,10], and it was suggested that absolute concentration of the far red-absorbing form of phytochrome is important [12]. In the phyB-deficient *phyB-1* mutant, *GA4H* expression is not induced by red light, although *GA4* expression

Figure 1

Gibberellin biosynthesis pathway. Abbreviations: CPS, copalyl diphosphate synthase; KS, *ent*-kaurene synthase; GA20ox, GA 20-oxidase; GA3h, GA 3β-hydroxylase; GA2h, GA 2β-hydroxylase (GA 2-oxidase). *ga1*, *ga2*, *ga3*, *ga4* and *ga5* are GA biosynthesis mutants of *Arabidopsis*.



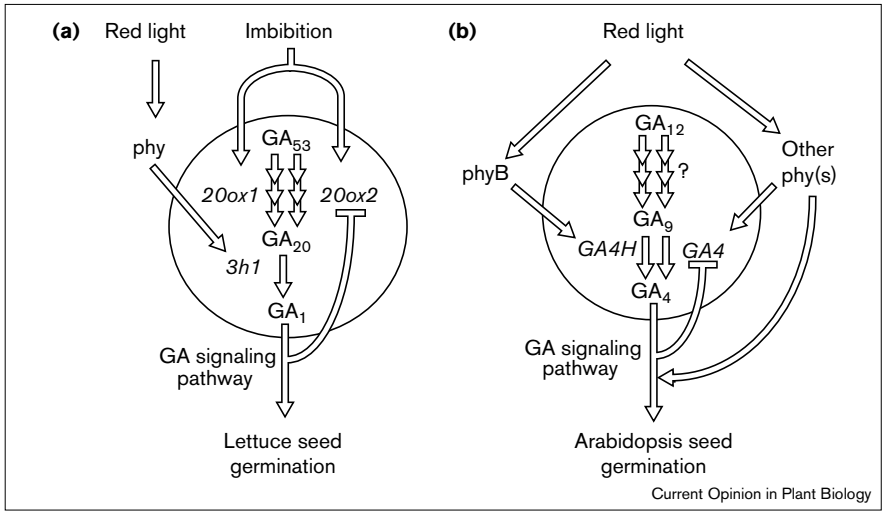
still is, indicating that the red light-induced *GA4* and *GA4H* expression is mediated by one or more other phytochromes (Figure 2b). In contrast to the *GA4* [21] and the *Ls3h1* [13••] genes, *GA4H* is not regulated by a feedback inhibition mechanism in germinating seeds [14••] (Note that *Ls3h1* gene is down-regulated by applied GA_1 but not by the elevated endogenous GA_1 level after red light). Although the endogenous GA levels of germinating *ga4* and wild-type (WT) seeds have not yet been analyzed, red light treatment is expected to increase the level of biologically active GAs. The two GA 3β-hydroxylases of *Arabidopsis*, therefore, seem to play different physiological roles during light-induction of seed germination (Figure 2b).

Regulation of GA biosynthesis by photoperiod

The involvement of GAs in the photoperiod-induced bolting of long-day (LD) rosette plants is well documented [1]. In spinach (*Spinacia oleracea*), changes in GA concentrations and enzyme activity in cell-free systems on transfer from short days (SD) to LD are consistent with enhanced oxidation of GA_{53} and GA_{19} in LD [22]. Furthermore, there are higher amounts of GA 20-oxidase mRNA in plants grown in LD than those in SD or in darkness [23]. Although GA_{53} 20-oxidase activity is regulated by light, oxidation of GA_{44}, in the lactone form, remains at high constant levels irrespective of the photoperiod. An expected difference between spinach GA_{44} oxidase and the recombinant *Arabidopsis* GA 20-oxidase was observed in the

Figure 2

Proposed models of the mechanism of GA control of seed germination in **(a)** lettuce and **(b)** *Arabidopsis*. Arrows indicate positive regulation. Feedback inhibition is shown by T-bar. In lettuce the two *Ls20ox* genes (*20ox1* and *20ox2*) are induced by imbibition. However, *20ox1* is not regulated by light. The *3h1* gene (coding for a GA 3β-hydroxylase) is feedback regulated by applied GA_1 but not by endogenous GA_1. In *Arabidopsis* there are two different GA 3β-hydroxylases (*GA4* and *GA4H*), both of which are controlled by phytochrome.



stereospecific removal of a hydrogen atom during oxidation of the C-20 alcohol intermediates [24]. This suggests the existence of a specific enzyme that catalyzes the oxidation of C-20 as a lactone. It might oxidize the alcohol from *in vivo*.

Phytochrome regulation of GA biosynthesis during seedling growth

Light inhibits stem elongation during photomorphogenesis [25], and the role that GAs (change of GA sensitivity and/or metabolism) play in that process has been the subject of a long-standing controversy [1]. Recently, work from two independent laboratories [26•] (Ait-Ali T *et al.*, Abstract at page 48, and Gil J, García-Martínez JL, Abstract at page 107, both at the 16th ICPGS, 10–16 August 1998, Chiba, Japan) have shown a rapid (within two hours) and reversible decrease of GA_1 content (down to trace level) in the apical shoot of etiolated pea seedlings upon light irradiation (Figure 3). The light, however, increases the transcript levels coding for GA 20-oxidase and GA 3β-hydroxylase in the apical shoot, indicating that they do not contribute to the decrease of GA_1 content induced by light. Work with *phyA*- and *phyB*-deficient pea mutants showed that the expression of GA 20-oxidase is regulated by both phyA and phyB [26•]. The increase in the transcript accumulation is probably the result of feedback inhibition due to the reduction of the GA_1 level, because it does not occur when the seedlings are treated with GA_1 before irradiation. The concentration of GA_8 — the inactive product of GA_1 metabolism — increases transiently in irradiated seedlings (Gil J, García-Martínez JL, Abstract at the 16th ICPGS, Japan), suggesting that GA 2β-hydroxylation may be regulated during de-etiolation. The recent isolation of clones coding for 2β-hydroxylases of *Phaseolus coccineus* and *Arabidopsis* [5••] should help to clarify this issue.

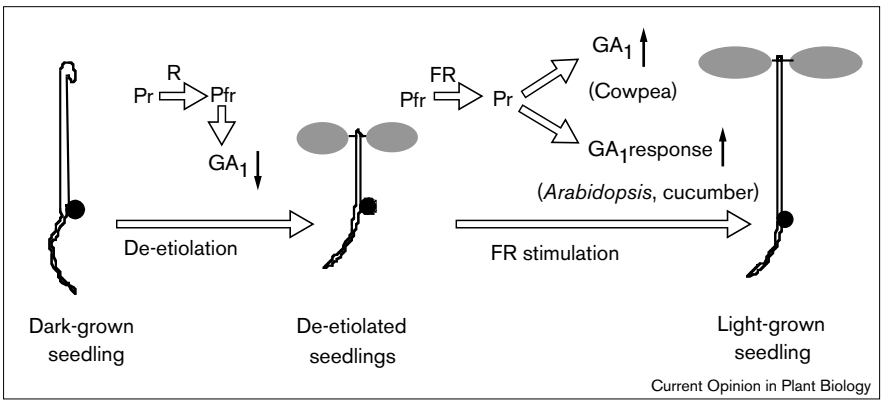
Treatment with end-of-day (EOD)-far red irradiation enhances stem elongation, and in cowpea also decreases [^{3}H]GA_1 inactivation [27] and increases GA_1 content in the elongating region of the epicotyl, but not in the leaves — an effect that can be reverted by subsequent red light treatment (JL García-Martínez, unpublished data) (Figure 3). This suggests that phytochrome may control stem elongation by regulating GA 2β-hydroxylation in light-grown seedlings. Work with the *Arabidopsis ga1 phyB* double mutant has shown that the full *phyB* mutant phenotype is expressed only in the presence of a completely functional GA system [28]. However, the role of phytochrome in the regulation of GA biosynthesis in light-grown plants is not clear. The overexpression of oat *PHYA* in tobacco [29] and hybrid aspen [30] decreases the content of active GAs and results in a short phenotype, that can be reversed by GA application. Potato plants overexpressing the antisense potato *PHYB* gene are taller and contain more GA_1 in the apical shoot (S Prat, personal communication). However, the *phyB* mutants of pea (*lv*), cucumber (*lh*) and *Arabidopsis*, which have an elongated phenotype, show no consistent differences in GA content compared to wild type [31–33]. Though some of these apparently contradictory results could be explained by the use of inappropriate plant materials for GA measurement (e.g. dilution of responding tissue by non-responding tissues), it seems clear that phytochrome regulates both GA biosynthesis and GA signaling [33,34] (Figure 3).

Low irradiance enhances stem elongation and increases the active GA levels in pea [35] and Brassica [36], an effect probably mediated by phytochrome. Low irradiance increases the transcript levels of GA 20-oxidase in pea leaves (JL García-Martínez, unpublished data), and in Brassica it reduces GA_1 and GA_8 conjugation [36]. Therefore, the effect of low irradiance on elongation seems to be due to an increase of GA_1 biosynthesis and to a decrease of GA_1 metabolism, in addition to an enhancement of responsiveness to GA_1, at least in pea [35].

Phytochrome regulation of GA biosynthesis during tuber formation in potato

Tuber formation in potato depends on temperature and nutrient conditions [7•]. It is inhibited by GA application, and promoted by genetic and chemical blocking of GA biosynthesis, suggesting that the process is regulated by GAs [37]. This hypothesis is supported by the observation

Figure 3



Role of phytochrome in the regulation of stem growth by GAs. In etiolated pea seedlings, the content of GA_1 is rapidly reduced by an still unknown mechanism. In light-grown seedlings, light can modulate stem elongation by changing its GA_1 content (e.g. EOD-FR enhancement of stem elongation associated with an increase of GA_1 in cowpea) or altering its GA responsiveness (e.g. *Arabidopsis* and cucumber).

that the content of GA_1 in the stolons is high when they are elongating, and decreases when they start to swell after transferring to inductive (high sucrose concentration) conditions [38•]. The photoperiod controls tuber formation, which is enhanced or absolutely dependent on short-day (SD) conditions (as in *Solanum tuberosum* ssp. *andigena*) [7•]. This photoperiod dependence is lost in anti-*PHYB* potato lines, which also form tubers under long-day (LD) conditions [39] (Figure 4). This indicates that phyB blocks tuber formation by producing an inhibitor under non-inductive LD, rather than by stimulating it under inductive SD. The photoperiod signal is perceived in the leaves, and grafting experiments have shown that wild-type leaves produce a graft-transmissible inhibitor of tuber formation, of unknown nature, that is absent in anti-*PHYB* plants under LD [40•]. However, although anti-*PHYB* transgenic potatoes form tubers under LD, as seen in wild-type plants treated with inhibitors of GA biosynthesis, they are taller than WT plants (as other *phyB* mutants), and have a higher GA_1 content in the apical shoot (S Prat, personal communication). This apparent paradox could be explained if phyB stimulated the transport of either a GA or a regulator of GA biosynthesis to the stolons under LD — without transport to the stolons the substance would accumulate in the leaves and thereby stimulate shoot elongation (Figure 4). Clearly, the identification and quantification of GAs separately in the leaves and stolons of wild-type and anti-*PHYB* potatoes are necessary to clarify the mechanism of phytochrome regulation and the possible mediation of GAs during tuber formation.

Three clones coding for GA 20-oxidases, that are also expressed in the stolons [41•], have recently been isolated from potato leaves (*StGA20ox1–3*). Under SD (inductive) conditions their transcript levels in the leaves fluctuate during the 24 h photoperiod and show a peak 4 h after the lights have been turned off. However, under non-inductive conditions (30 min light-break during the dark period) a second peak of *StGA20ox1* and *StGA20ox3* transcript levels is observed later in the night. *StGA20ox1*, which is strongly expressed in the leaves, may thus play a role in the observed accumulation of GA_1 in the shoot of anti-*PHYB* potato. The role of these genes in the control of tuber formation, however, is unclear, since nothing is known about the phyB regulation of their expression in the stolons. However, the observation that in LD tuber formation in a potato dwarf mutant with a blockage in GA biosynthesis, and in transgenic anti-GA 20-oxidase potatoes takes much longer than in wild-type potatoes under SD (S Prat, personal communication) suggests that other factors in addition to GAs are also involved.

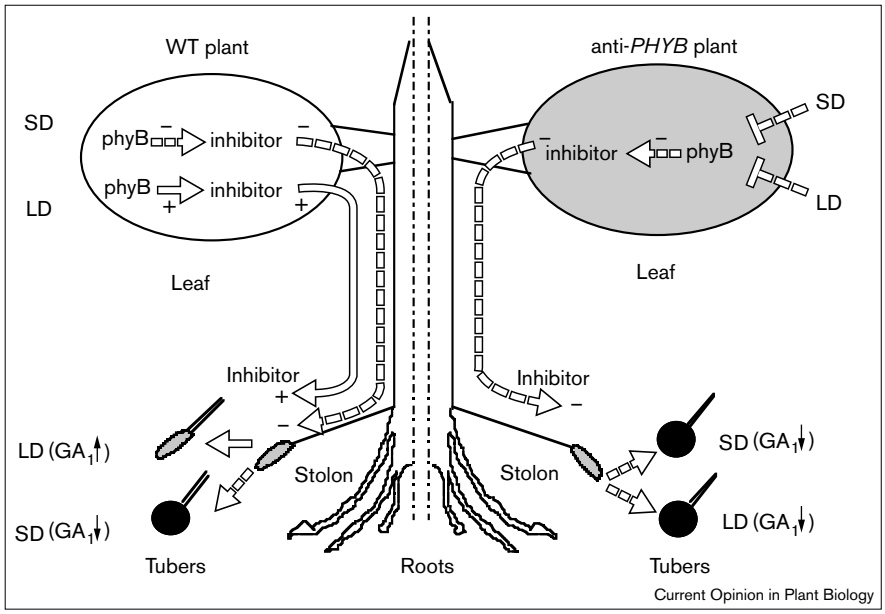
Diurnal regulation of GA biosynthesis

The content of GAs changes diurnally [1], though the meaning of these changes is still unclear. Sorghum is a SD plant, and the *phyB* mutation advances the peaks of GA_{20} and GA_1 and induces flowering in LD [42]. In wild-type plants, short photoperiods that induce flowering also advance the GA_{20} and GA_1 maxima, as in *phyB* mutants under LD [43•]. Thus, phyB seems to control the daily regulation of GA_{20} biosynthesis in sorghum. This hypothesis has received recent support from work with potato. In this species (ssp. *andigena*) tuber formation is regulated by phyB (Figure 4), and LD conditions, that prevent or delay tuberization, induce additional peaks of *StGA20ox* transcripts in the leaves, compared to plants grown under inductive SD conditions [41•].

Conclusions

Germination of lettuce and *Arabidopsis* is regulated by phytochrome and this is at least mediated by regulation of GA

Figure 4



Proposed model for the role of phyB in the regulation of tuber formation by GAs. In WT plants containing phyB (left) LD (continuous lines) stimulates, whereas SD (dashed lines) prevents the synthesis and/or transport of an inhibitor of tuber formation from the leaves to the stolons, where it induces the accumulation of GA_1 inhibiting tuber formation. The nature of the inhibitor is still unknown (e.g. GA_1 itself, a precursor of GA_1 or an activator of GA_1 biosynthesis in the stolons). In anti-*PHYB* plants (right) LD can not stimulate the synthesis and/or transport of the inhibitor, therefore tuber formation is independent of photoperiod. The anti-*PHYB* plants contain more GA_1 in the shoot and are taller, probably due to the accumulation in the leaves of the inhibitor as a result of its transport blockage to the stolons, therefore favoring tuber formation.

biosynthesis. The levels of GA 3β-hydroxylase transcripts and endogenous GAs, at least in lettuce (GAs have not been measured in *Arabidopsis* yet) are regulated by phytochromes, but the mechanism to increase active GAs in germinating seeds is probably different between them. During the de-etiolation of pea seedlings, the endogenous GA_1 content in the apical shoot decreases drastically within two hours of the plant being transferred from dark to light. However, the drop of GA_1 does not correlate well with the changes of GA 20-oxidase and GA 3β-hydroxylase gene expression induced by phytochrome, suggesting that other GA biosynthetic genes are involved in the process. Photoperiod is perceived by phyB in potato leaves, from where an inhibitor of tuber formation seems to be transported to the stolons under non-inductive conditions. The relationship between this inhibitor and GAs, which are also inhibitors of tuber formation, is still unknown.

Acknowledgements

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This short communication shows, by using grafting experiments between antisense phyB (anti-*PHYB*) and wild-type potato plants, that phyB controls the production in the leaves of a graft-transmissible inhibitor of tuber formation. The anti-*PHYB* plants tuberize under non-inductive conditions because they produce or transport less inhibitor.

41. Carrera E, Jackson SD, Prat S: **Feedback control and diurnal**
• **regulation of gibberellin 20-oxidase transcript levels in potato.** *Plant Physiol* 1999, **119**:765-773.

Three GA 20-oxidases were cloned from potato, all being expressed in the stolons and one of them (*StGA20ox1*) at high levels in the leaves. It was found that the content of *StGA20ox* transcripts in the leaves follows a diurnal rhythm under inductive (SD) conditions of tuberization. Under non-inductive (30 min night-break or LD) conditions another peak of *St20ox1* and *St20ox2* transcripts appears, suggesting that the diurnal rhythm of endogenous GAs may play a role in the control of tuberization.

42. Foster KR, Morgan PW: **Genetic regulation of development in *Sorghum bicolor*. IX. The ma_3^R allele disrupts diurnal control of gibberellin biosynthesis.** *Plant Physiol* 1995, **108**:337-343.

43. Lee IJ, Foster KR, Morgan PW: **Photoperiod control of gibberellin**
• **levels and flowering in sorghum.** *Plant Physiol* 1998, **116**:1003-1011.

This paper confirms and extends previous results on the existence of rhythmic peaks in levels of endogenous GAs in wild-type and *phyB* sorghum, a SD plant. The patterns of GA_1 and GA_{20} peaks in wild-type plants grown under inducing conditions were similar to those of *phyB* plants, which flowers almost independently of photoperiodic conditions. The photoperiod also affects the pattern of GA content in *phyB* plants associated to its effect on flower initiation.