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Interaction of light and hormone signals in germinating seeds

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Abstract Seed germination is regulated by several environmental factors, such as moisture, oxygen, temperature, light, and nutrients. Light is a critical regulator of seed germination in small-seeded plants, including Arabidopsis and lettuce. Phytochromes, a class of photoreceptors, play a major role in perceiving light to induce seed germination. Classical physiological studies have long suggested the involvement of gibberellin (GA) and abscisic acid (ABA) in the phytochrome-mediated germination response. Recent studies have demonstrated that phytochromes modulate endogenous levels of GA and ABA, as well as GA responsiveness. Several key components that link the perception of light and the modulation of hormone levels and responsiveness have been identified. Complex regulatory loops between light, GA and ABA signaling pathways have been uncovered.

Keywords Abscisic acid · Gibberellin · Phytochrome · Seed germination

Introduction

Upon the completion of embryogenesis, the embryo within a seed stops growth and becomes dormant. The dormant seeds do not germinate and can survive under changing temperature and moisture conditions for a long time. Dormancy breaks down after a period of storage and seeds germinate under a certain condition. Seeds are equipped with sophisticated sensors and machineries to monitor and determine whether the surrounding environment is suitable for germination and subsequent plant growth. For small-seeded plants including Arabidopsis, lettuce, tomato and tobacco, light is an important regulator of seed germination. If germinated deep underground, the seedlings with small nutrient reserves cannot reach the surface of soil due to lack of energy. Thus, light sensing by the seed is thought to play a role in detecting its position. Phytochromes are the major class of photoreceptors responsible for this process. Light signals received by phytochromes are converted to internal cues, which in turn regulate physiological processes in seeds. Gibberellin (GA) and abscisic acid (ABA) are the internal signals that play central roles in the regulation of seed germination; GA induces, whereas ABA inhibits, seed germination. Recent studies have begun to reveal a tight interaction between light, GA and ABA signaling pathways in seeds at the molecular level.

In this review, we first describe a brief overview of each pathway, and then discuss how light and hormone signals interact to control seed germination. Here, we mainly focus on Arabidopsis, but also briefly discuss distinct as well as common mechanisms in lettuce and Arabidopsis during light-induced seed germination.

Phytochromes

Plants have at least four different types of photoreceptors; the red (R)/far-red (FR) light photoreceptor phytochromes, the UV-A/blue light photoreceptor cryptochromes, the blue light receptor phototropins, and the newly discovered blue

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light photoreceptors zeitlupes (Bae and Choi 2008). Phytochromes were originally discovered based on the photo-reversible germination of lettuce seeds. Irradiation of dark-imbibed seeds with a R light pulse induces germination and a subsequent FR light pulse cancels the effect of R light (Borthwick et al. 1952). Two forms of phytochromes, Pr and Pfr (the R light and FR light absorbing forms, respectively) exist in plants. Pr is an inactive form and is converted to active Pfr by R light. Pfr is reversibly converted to Pr by FR light (Fig. 1). This is the typical photo-reversible regulation of light response by phytochromes. The conformational change is accompanied by the reversible targeting of Pfr into the nucleus, where it binds to interacting proteins and regulates various physiological processes including seed germination.

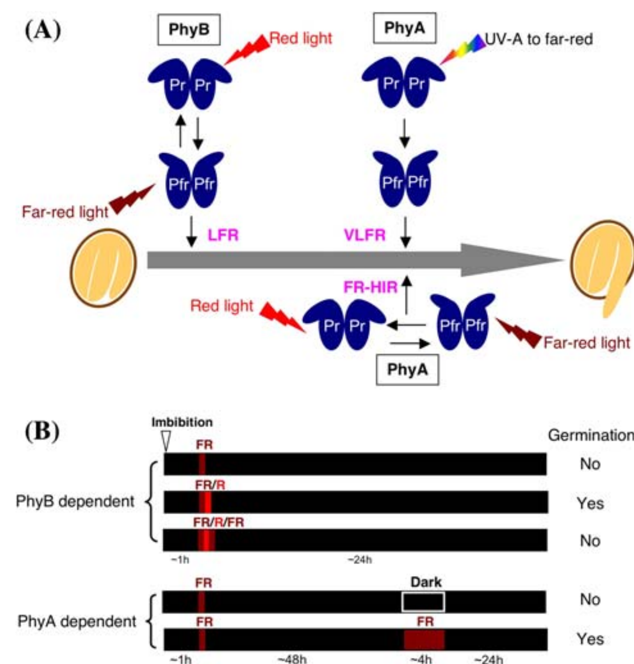

Fig. 1 **a** Modes of phytochrome mediated seed germination in Arabidopsis. PhyB, the major phytochrome accumulated in seeds, mediates typical R/FR photoreversible response (LFR) to promote seed germination in the early phase of seed imbibition. After a prolonged imbibition in the dark, PhyA is accumulated in the seeds and promote seed germination in response to either very low fluence of wide range of wavelength from UV-A to FR (VLFR) or relatively longer irradiation of FR light (FR-HIR). Phytochromes are dimeric proteins typically consisting of two identical apoproteins. **b** Light treatments for PhyA- and PhyB-dependent germination assay in Arabidopsis seeds. For PhyB-dependent germination assay, R and FR light pulses ($10^{-7} \sim 10^{-4}$ mol m^{-2}) are irradiated shortly after imbibition ($\sim$1 h) to reversibly control germination. Germination (radicle emergence) is normally observed within 24 h after PhyB activation. For PhyA-dependent germination assay, a FR light pulse is first irradiated to inactivate PhyB at the beginning of imbibition. After a long period of dark incubation ($\sim$48 h), FR light ($\sim$4 h, $>10^{-2}$ mol m^{-2}) is irradiated to activate PhyA. Seed germination can be also observed within 24 h of PhyA activation

Phytochromes are encoded by a multigene family in most plant species. In Arabidopsis, there are five *PHY* genes encoding phytochrome apoproteins (*PHYA* to *PHYE*) (Mathews 2006). PhyB (holoprotein), mediating R/FR photoreversible low fluence responses (LFR), promotes seed germination in response to low R light during initial stage (up to several hours) of seed imbibitions, whereas PhyA, mediating photo-irreversible very low fluence response (VLFR) and FR high irradiance response (FR-HIR), promotes seed germination in response to very low fluence of relatively wide spectra of light and continuous FR light after longer imbibition period in the absence of active PhyB in the dark (Shinomura et al. 1994, 1997; Fig. 1). In addition, PhyE has been shown to mediate LFR and FR-HIR once seeds are imbibed. The typical R/FR photo-reversible germination in lettuce corresponds to LFR.

GA pathways

Endogenous hormone levels are determined by the rates of their formation and conversion into inactive forms. Here, we refer to biosynthesis as the production of bioactive forms of a hormone, deactivation as conversion of bioactive forms (and their precursors) to the inactive (or less active) forms, and metabolism as both biosynthesis and deactivation.

GAs are a group of diterpenoids that are biosynthesized from geranylgeranyl diphosphate (GGDP) (Fig. 2). Among more than 100 GAs identified from plants, only a small number of them, such as GA_1 and GA_4, act as bioactive hormones that control seed germination, leaf expansion, stem elongation and flowering (Yamaguchi 2008). The late steps in the GA biosynthetic pathway are catalyzed by GA 20-oxidase (GA20ox) and GA 3-oxidase (GA3ox), both of which are 2-oxoglutarate-dependent dioxygenases (2ODDs) (Fig. 2). GA20ox and GA3ox are each encoded by small multigene families (Fig. 2). Accumulating evidence indicates that these 2ODDs are the primary targets for the regulation of bioactive GA levels (Yamaguchi 2008). GAs are deactivated by multiple classes of enzymes. The best-characterized GA deactivation enzyme is GA 2-oxidase (GA2ox), which is also a class of 2ODDs (Fig. 2). Recently, studies have shown that GA 16, 17-epoxydase (GA16,17ox) (CYP714D1/ELONGATED UPPERMOST INTERNODE) from rice and GA methyltransferases (GAMT1 and GAMT2) from Arabidopsis also act as GA-deactivating enzymes (Yamaguchi 2008; Fig. 2).

Several components in the GA signaling pathways have been identified. DELLA proteins (named after an amino acid sequence in a conserved domain) are members of the GRAS protein family acting as negative regulators in the GA signaling pathway (Sun and Gubler 2004). A soluble

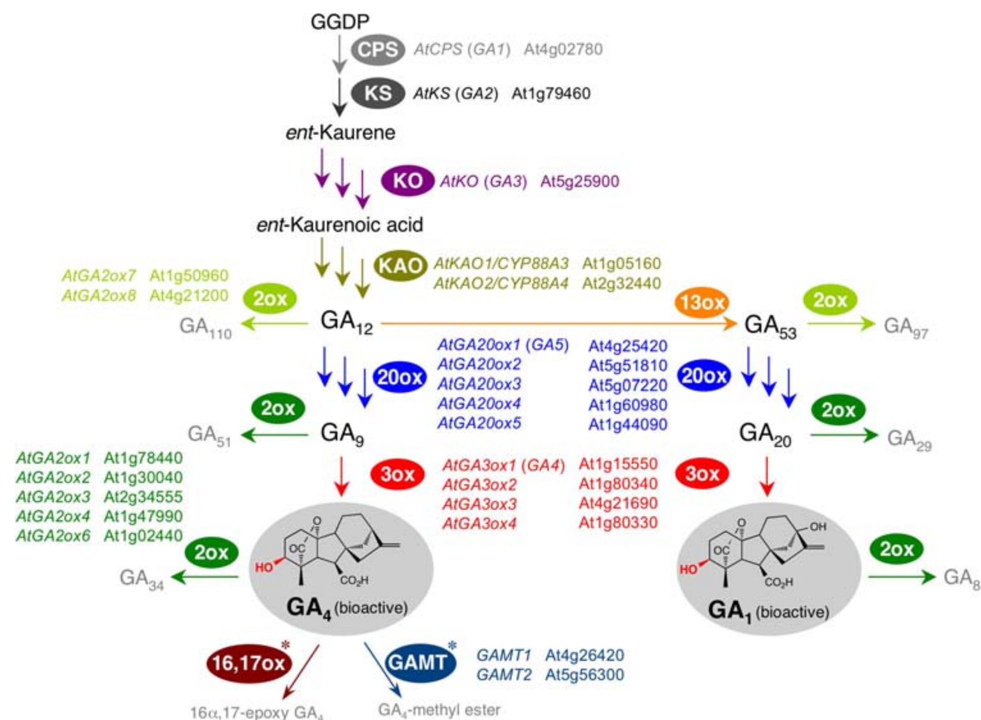

Fig. 2 The GA metabolic pathway. In germinating Arabidopsis seeds, GA_4 is the major bioactive GA. Inactivated GAs are shown in grey letters. Enzymes, genes (locus) and AGI codes are shown. See Yamaguchi (2008) for details. 2ox, GA 2-oxidase; 3ox, GA 3-oxidase; 20ox, GA 20-oxidase; 16,17ox, GA 16α,17-epoxidase, GAMT, GA methyltransferase; GGDP, geranylgeranyl diphosphate; CPS, *ent*-copalyl diphosphate synthase; KS, *ent*-kaurene synthase; KO, *ent*-kaurene oxidase; KAO, *ent*-kaurenoic acid oxidase. *16,17ox and GAMT also use other GAs as substrates, which are not shown for simplification

GA receptor, GIBBERELLIN INSENSITIVE DWARF 1 (GID1) in rice and its Arabidopsis orthologs, AtGID1a, b and c, interact with DELLA proteins in the presence of bioactive GAs (Ueguchi-Tanaka et al. 2007). The GA-GID1-DELLA complex is recognized by an F-box protein (SLEEPY1 [SLY1] in Arabidopsis and GID2 in rice) (McGinnis et al. 2003; Sasaki et al. 2003) for ubiquitination of the DELLA protein, which is then degraded through the 26S proteasome pathway. This results in de-repression (activation) of the GA signaling pathway (Fig. 3; Ueguchi-Tanaka et al. 2007; Hirano et al. 2008; Schwechheimer 2008). Genetic evidence has proven the role of these three components in regulating seed germination in Arabidopsis. Loss-of-function mutations in *DELLA* genes lead to increased germination capacity (Lee et al. 2002; Cao et al. 2005), whereas mutants defective in the SLY1 F-box protein or the AtGID1 receptors are non-germinating (Steber et al. 1998; Griffiths et al. 2006; Iuchi et al. 2007; Willige et al. 2007).

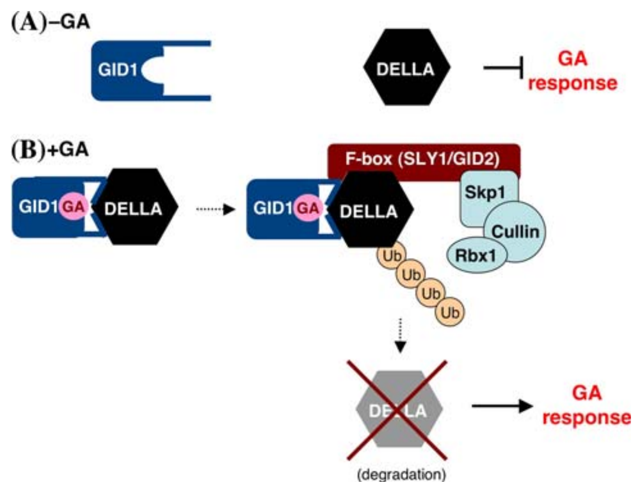

Fig. 3 Model for GA action through degradation of DELLA proteins. **a** In the absence of GA, DELLA proteins are stable and negatively regulate the GA response pathway. **b** Once GA binds the GA receptor GID1, the GID1-GA complex interacts with DELLA protein. The GA-GID1-DELLA complex is recognized by an F-box protein (SLY1 in Arabidopsis and GID2 in rice) for ubiquitination (Ub) in the SCF complex (containing Skp1, Cullin and Rbx1). The ubiquitinated DELLA protein is degraded through the 26S proteasome, which results in de-repression of the GA response pathway

ABA pathways

ABA is a sesquiterpenoid derived from carotenoids and is involved in the induction of stress-responsive genes, stomatal closure, seed development, dormancy and germination (Nambara and Marion-Poll 2005). The ABA metabolic pathway is outlined in Fig. 4. Mutants defective in an earlier step of carotenoid synthesis exhibit an albino phenotype in addition to ABA deficiency. In contrast, mutants defective in zeaxanthin epoxidase (ZEP) show typical phenotypes due to ABA deficiency. There is evidence that the step catalyzed by 9-*cis* epoxycarotenoid dioxygenase (NCED) is limiting the level of ABA in the biosynthesis pathway (Nambara and Marion-Poll 2005). It has recently been reported that ABA is also produced via hydrolysis of ABA glucose ester by ABA glucosiodase,

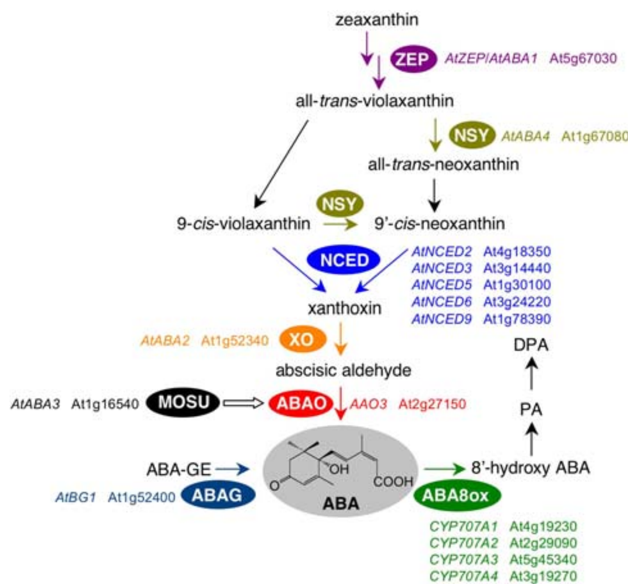

Fig. 4 The ABA metabolic pathway. Enzymes, genes and AGI codes are shown. See Nambara and Marion-Poll (2005); Lee et al. (2006) and North et al. (2007) for details. ABA8ox, ABA 8′-hydroxylase; ABAG, ABA glucosidase; ABA-GE, ABA glucose ester; ABAO, abscisic aldehyde oxidase; DPA, dihydro phaseic acid; MOSU, molybdenum cofactor sulfurase; NCED, nine-*cis*-epoxycarotenoid dioxigenase; NSY, neoxanthin synthase; PA, phaseic acid; XO, xanthoxin oxidase; ZEP, zeaxanthin epoxidase

AtBG1, in Arabidopsis (Lee et al. 2006; Fig. 4). A major route for ABA deactivation is hydroxylation of the C-8′ position to produce 8′-hydroxy ABA by ABA 8′-hydroxylase, a class of P450 s designated as CYP707As. 8′-hydroxy ABA is subsequently converted to phaseic acid and dihydrophaseic acid (Nambara and Marion-Poll 2005; Fig. 4).

Three possible ABA receptors, the RNA binding protein FCA, the Mg-chelatase H subunit CHLH/ABAR/GUN5 and the G protein-coupled receptor GCR2, have been reported to date (Wang and Zhang 2008). Among these, CHLH/ABAR/GUN5 appears to function in seeds because a loss-of-function mutation in this gene results in ABA-insensitive germination (Shen et al. 2006). ABA signaling has been extensively reviewed elsewhere (Finkelstein et al. 2002, 2008; Hirayama and Shinozaki 2007) and will not be discussed here in detail. Future work will need to elucidate how ABA signaling components are tied to regulate germination.

Regulation of GA metabolism by light

Light promotes germination of small-seeded plants, such as Arabidopsis and lettuce via phytochromes (Shinomura 1997). R right that induces germination can be substituted by an application of bioactive GA to lettuce seeds (Kahn et al. 1957). In addition, R light cannot induce seed germination of severe GA-deficient mutants, such as *ga1*-3, in Arabidopsis (Oh et al. 2006). These results suggest that phytochromes promote germination by regulating GA levels in seeds. Light-regulation of GA metabolism has been studied in detail under PhyB-dependent or related germination conditions in Arabidopsis and lettuce seeds, where endogenous levels of bioactive GAs increase after irradiation with a R-light pulse and the increase is suppressed by a FR-light pulse given immediately after the R-light pulse (Toyomasu et al. 1993; Oh et al. 2006; Seo et al. 2006; Fig. 5). Consistent with the change in GA levels, expression of GA biosynthetic genes encoding GA3ox (*AtGA3ox1* and *AtGA3ox2* in Arabidopsis and *LsGA3ox1* in lettuce) is induced by R light and the induction is canceled by FR light (Toyomasu et al. 1998; Yamaguchi et al. 1998; Fig. 5). In contrast, transcript levels of a GA deactivating gene *GA2ox* (*AtGA2ox2* in Arabidopsis and *LsGA2ox2* in lettuce) are decreased by R light treatment (Nakaminami et al. 2003; Oh et al. 2006; Seo et al. 2006; Yamauchi et al. 2007; Fig. 5). Light-regulation of *GA3ox* and *GA2ox* genes are also observed during PhyA-dependent germination (Oh et al. 2006). These data demonstrate that bioactive GA levels are regulated by phytochromes through reciprocal regulation of *GA3ox* and *GA2ox* genes.

Regulation of ABA metabolism by light

ABA treatment inhibits germination of lettuce seeds induced by R light or by exogenous application of bioactive GAs (Khan 1968; Sankhla and Sankhla 1968). More recently, ABA-deficient mutant seeds have been shown to partially germinate even in the absence of inductive light in Arabidopsis (Seo et al. 2006). Thus, endogenous ABA levels might also be controlled by light. In fact, R light decreases ABA levels through LFR in Arabidopsis and lettuce seeds (Toyomasu et al. 1994; Seo et al. 2006; Oh et al. 2007; Fig. 5). In Arabidopsis seeds, ABA levels decrease at the initial phase of imbibition regardless of light treatment. However, the rates of decrease at the later phase of imbibition are modulated by light; FR light-treated seeds contain higher levels of ABA than do FR/R light-treated seeds (Seo et al. 2006; Fig. 5). Consistent with the change in ABA levels, expression levels of ABA biosynthetic genes encoding ZEP (*AtZEP/AtABA1* in Arabidopsis) and NCED (*AtNCED6* and *AtNCED9* in Arabidopsis and *LsNCED2* and *LsNCED4* in lettuce) are decreased by R light treatment (Seo et al. 2006; Oh et al. 2007; Sawada et al. 2008a; Fig. 5). In contrast, transcript levels of ABA deactivating genes encoding CYP707A (*CYP707A2* in Arabidopsis and *LsABA8ox4* in lettuce) are

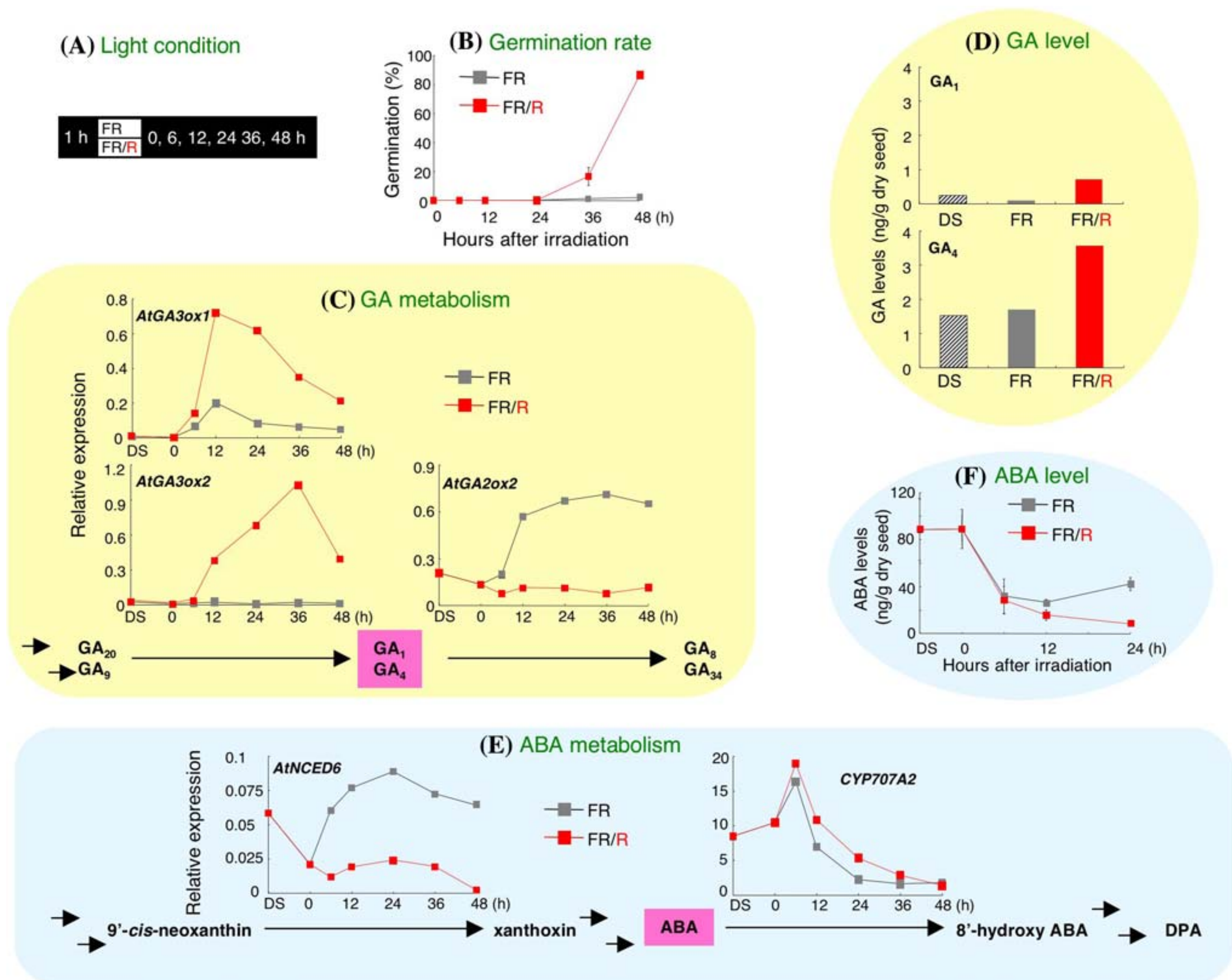

Fig. 5 Regulation GA and ABA metabolism during phytochrome-dependent germination in Arabidopsis. **a** Light treatments for PhyB-dependent germination. **b** Germination profiles. **c** Expression profiles of GA biosynthetic genes (*AtGA3ox1* and *AtGA3ox2*) and a GA deactivating gene (*AtGA2ox2*). **d** Endogenous levels of bioactive GAs. **e** Expression profile of an ABA biosynthetic gene (*AtNCED6*) and an ABA deactivating gene (*CYP707A2*). **f** Endogenous ABA levels. DS, dry seeds. [Modified from Seo et al. (2006).]

elevated by R light treatment (Seo et al. 2006; Oh et al. 2007; Sawada et al. 2008a; Fig. 5). In lettuce, a decrease in ABA levels is accompanied by an increase in phaseic acid levels upon R light treatment (Sawada et al. 2008a). These results indicate that ABA concentrations in seeds are regulated by phytochrome in a manner opposite to GA concentrations.

Interaction between GA and ABA metabolism

Unlike wild type, ABA-deficient mutants including *aba2*, *aao3* and *nced6* partially germinate even in the absence of R light treatment (Seo et al. 2006). Likewise, treatment with fluridone (an ABA biosynthesis inhibitor) increases the germination frequency in the absence of R-light. The *aba1* mutation allows *ga1* mutant seeds to germinate under continuous white light, suggesting that GA biosynthesis is not required for germination in an ABA-deficient mutant background (Koornneef et al. 1982). In comparison, the germination of *aba2* seeds under the non-inductive light condition is inhibited by a GA biosynthesis inhibitor paclobutrazol, suggesting that de novo GA biosynthesis causes the germination (Seo et al. 2006). In fact, the levels of GA_4 and expression of GA biosynthetic genes (*AtGA3ox1* and *AtGA3ox2*) are elevated in the *aba2* mutant compared to those in wild type. Furthermore, the PhyB-mediated induction of these GA biosynthetic genes is partly suppressed in an ABA over-accumulating *cyp707a2* mutant (Seo et al. 2006). Altogether, these data show that bioactive GA levels are negatively regulated by ABA during seed germination (Seo et al. 2006).

There is also evidence that GA negatively regulates endogenous ABA concentrations. In Arabidopsis, higher ABA levels, activation of ABA biosynthetic genes and suppression of an ABA deactivating gene are observed in the GA deficient mutant *ga1* in comparison with wild type (Oh et al. 2007). Moreover, an application of bioactive GA to *ga1* seeds under non-inductive light condition decreases and increases expression levels of ABA biosynthetic and ABA deactivating genes, respectively (Oh et al. 2007). Similarly, treatment of lettuce seeds with bioactive GA decreases ABA content and *LsNCED4* transcript levels in the absence of Pfr (Toyomasu et al. 1994; Sawada et al. 2008a). Zentella et al. (2007) reported that *XERICO*, one of the early GA- and DELLA-responsive genes, regulated ABA levels in seeds. *XERICO* encodes a protein containing a RING (really interesting new gene)-H2 type zinc finger motif. Overexpression of this gene causes accumulation of ABA and enhanced drought tolerance in vegetative tissues presumably through an increased ABA biosynthesis (Ko et al. 2006). Chromatin immunoprecipitation (ChIP) analysis indicates that DELLA proteins repress the expression of *XERICO* by directly associating with its promoter (Zentella et al. 2007). These results indicate that the repression of *XERICO* by DELLA proteins is an important mechanism by which GA regulates ABA metabolism. The mutual negative regulation between GA and ABA might contribute to an efficient change in the balance of these antagonistic hormones in response to an external cue (Fig. 6).

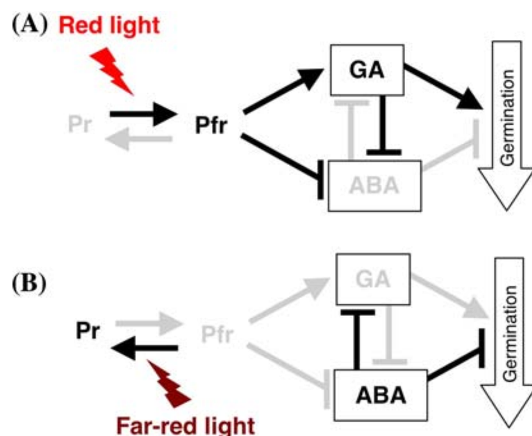

Fig. 6 Interaction of ABA and GA metabolism. **a** Pfr formed by R light increases GA levels and decreases ABA levels. R-light induced GA accumulation also contributes to reducing ABA levels. **b** In the absence of Pfr, ABA levels are higher because of the loss of negative regulation by Pfr. Increased ABA levels are required to reduce GA levels. Arrows indicate positive regulation and T-bars indicate negative regulation. Grey elements denote inactive regulation or low abundance in each condition

Factors that link light signal to GA and ABA signals

PIL5

Phytochromes modulate GA and ABA metabolism through one of their interacting proteins, PIL5 (PIF3–LIKE 5). PIL5 is a phytochrome-interacting basic helix-loop-helix (bHLH) protein that belongs to the Arabidopsis bHLH subfamily 15 together with PIF3 (PHYTOCHROME INTERACTING FACTOR 3), PIF4, PIL6, and 10 other bHLH proteins (Toledo-Ortiz et al. 2003; Yamashino et al. 2003). PIL5 preferentially interacts with the Pfr form of PhyA and PhyB, and the interaction induces the degradation of PIL5 protein through the 26S proteasome (Oh et al. 2004, 2006). Loss-of-function *pil5* mutant seeds exhibit constitutive germination even under non-inductive light condition, whereas the PIL5 overexpressor requires the higher fluence of light for seed germination. Taken together, the results indicate that phytochromes promote seed germination by activating the degradation of PIL5 (Oh et al. 2004).

Gene expression analysis and quantification of hormone levels indicate that PIL5 regulates both GA and ABA levels by modulating expression of their metabolic genes (Fig. 7). Expression of two GA biosynthetic genes (*AtGA3ox1* and *AtGA3ox2*) is activated, whereas a GA deactivating gene (*AtGA2ox2*) is suppressed, in the *pil5* mutant (Penfield et al. 2005; Oh et al. 2006). Consistent with gene expression patterns, endogenous GA_4 levels are increased in the *pil5* mutant compared to wild type (Oh et al. 2006; Fig. 7). ABA biosynthetic genes (*AtABA1*, *AtNCED6* and *AtNCED9*) are highly expressed in FR light-irradiated seeds but repressed in the R light-irradiated

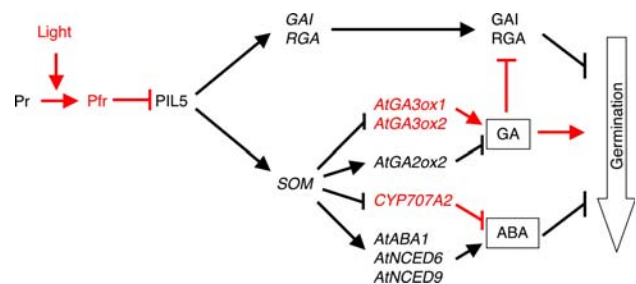

Fig. 7 Regulation of GA and ABA signaling by PIL5 and SOM. PIL5 is a negative regulator of seed germination. PIL5 protein is degraded upon interaction with active phytochrome. PIL5 acts as a transcriptional activator that binds directly to the promoter of *SOM*, *GAI* and *RGA*. SOM negatively regulates seed germination in part through regulation of GA and ABA levels. GAI and RGA are Arabidopsis DELLA proteins that act as negative regulators in the GA response pathway. GAI and RGA proteins are further degraded by GA activity (see Fig. 3). Arrows indicate positive regulation and T-bars indicate negative regulation. Active elements and regulation in response to inductive light treatment are shown in red

seeds. In contrast, an ABA deactivating gene (*CYP707A2*) is upregulated by R light irradiation. These regulations are also observed in the *ga1* mutant background, suggesting that the changes in gene expression are not secondary effects accompanied by germination. In contrast to wild-type and *ga1* mutant seeds, ABA biosynthetic genes are constitutively repressed in the *pil5 ga1* double mutant irrespective of light condition, while an ABA deactivating gene is expressed higher. Consistent with gene expression patterns, endogenous ABA levels are constitutively low in the *pil5* mutant background irrespective of light conditions. Taken together, the results indicate that PIL5 reciprocally regulates GA and ABA levels by regulating the expression of GA and ABA metabolic genes (Oh et al. 2007; Fig. 7).

Light also enhances GA responsiveness in imbibed Arabidopsis seeds via phytochromes (Hilhorst and Karssen 1988; Derkx and Karssen 1993; Yang et al. 1995; Oh et al. 2007). Germination frequency of a severe GA-deficient *ga1* mutant is increased by inductive light treatment in response to exogenous GA. However, the *pil5 ga1* double mutant constitutively shows high GA responsiveness and R light does not further increase the GA responsiveness, suggesting that PIL5 regulates the GA responsiveness as well as GA metabolism (Oh et al. 2007). Arabidopsis has five genes encoding DELLA proteins (RGA, GAI, RGL1, RGL2 and RGL3), negative regulators in the GA response pathway (Fig. 3). The increase in GA responsiveness by light is likely due in part to the transcriptional repression of two *DELLA* genes (*RGA* and *GA1*). Transcript levels of *RGA* and *GAI* decrease when PhyA or PhyB is activated even in the *ga1* mutant background. In the *pil5* mutant seeds, their expression levels are constitutively very low. The double-loss-of-function mutant of *GAI* and *RGA* (*gai-t6 rga-28*) is hypersensitive to R light for seed germination, supporting the functional significance of the two *DELLA* genes in repressing seed germination. In contrast to Arabidopsis, however, GA signaling is not affected by light in lettuce seeds, and expression of genes encoding DELLA proteins is not significantly affected by light (Sawada et al. 2008b).

The double loss-of-function *gai-t*6 *rga*-28 mutant, though hypersensitive to light, still requires light for seed germination, whereas the triple loss-of-function mutant of *GAI*, *RGA*, and *RGL2* germinates irrespective of light condition, indicating that light must inactivate RGL2 to promote seed germination (Tyler et al. 2004; Cao et al. 2005). Since GA activates the degradation of DELLA proteins, the increased bioactive GA level by light is likely to decrease the amount of RGL2 protein in light-treated seeds. Therefore, the results indicate that phytochrome and PIL5-mediated light signaling activates GA signaling both by repressing transcription of two *DELLA* genes and by increasing bioactive GA levels in imbibed seeds (Fig. 7).

ChIP analysis has shown that PIL5 binds to promoters of *RGA* and *GAI* genes, but does not bind to promoters of any GA and ABA metabolic genes, indicating that PIL5 directly regulates GA signaling genes but indirectly regulates metabolic genes. However, *GAI* and *RGA* are not the major PIL5 direct target genes mediating the regulation of GA and ABA metabolic genes. Expression of all GA and ABA metabolic genes tested, except for *AtGA3ox1*, is similarly regulated between wild type and the *gai-t6 rga-28* double loss-of-function mutant seeds in response to light (Oh et al. 2007). The expression of *AtGA3ox1* is still induced by light in the double mutant, but the expression level is relatively higher in the double mutant compared to wild type. Taken together, the results suggest that the regulation of GA and ABA metabolic genes by PIL5 is largely independent of GAI and RGA (Oh et al. 2007).

SOM

The indirect regulation of *GA* and *ABA* metabolic genes by PIL5 independently of *GAI* and *RGA* suggests that other PIL5 direct target genes regulate the expression of the metabolic genes. Recent analysis of *somnus* (*som*) mutant suggests that SOM regulates *GA* and *ABA* metabolic genes downstream of PIL5. The *som* mutant was isolated based on its ability to germinate under complete darkness (Kim et al. 2008). The *SOM* gene encodes a CCCH-type zinc finger protein that probably functions as an RNA binding protein. The *som* mutant contains higher levels of GA_4 and lower levels of ABA, with consistent changes in expression levels of *GA* and *ABA* metabolic genes. ChIP analysis indicates that PIL5 directly binds to the promoter of *SOM* and activates the expression of it. Taken together, these data suggest that PIL5 regulates *GA* and *ABA* metabolic genes partly through SOM (Kim et al. 2008; Fig. 7). How SOM regulates these metabolic genes needs to be further investigated. Unlike PIL5, SOM does not regulate the expression of *DELLA* genes, and GA responsiveness appears to be normal in *som* mutant seeds (Kim et al. 2008; Fig. 7).

IMB1

In addition to PIL5 and SOM, IMBIBITION-INDUCIBLE1 (IMB1) is implicated to play a role in linking PhyA signaling to ABA signaling. IMB1 is a member of the bromodomain protein family, which probably function as transcription factors. *IMB1* expression levels are very low in dry seeds and elevated upon imbibition (Duque and Chua 2003). A loss-of-function *imb1* mutant is defective in PhyA-dependent seed germination and hypersensitive to exogenous ABA in terms of inhibition of cotyledon greening during seed germination. The mutant phenotypes

can be partially explained by the higher accumulation of *ABI5* transcripts in response to exogenous ABA during germination (Duque and Chua 2003). The exact molecular mechanism by which IMB1 mediates the PhyA signaling during seed germination needs to be further investigated.

Conclusions and future perspectives

It has long been suggested that the balance of GA and ABA levels determines the germination capacity. This theory is supported by the fact that ABA-deficient mutants have been found as suppressors of non-germinating GA-deficient mutants (Koornneef et al. 1982; Bentsink and Koornneef 2002). This means that the amount of GA required for seed germination is decreased when endogenous ABA levels are reduced. Thus, the germination capacity is not determined by the levels of one hormone alone, but by the balance of the two antagonistic hormones. This hormone balance theory predicts that it should be an efficient way for seeds to regulate both GA and ABA levels in response to a signal in making a decision to germinate or not. Recent studies have proven that this is actually the case for light-regulation of seed germination in Arabidopsis, as discussed here. Light regulates concentrations of two antagonistic hormones, GA and ABA, and also GA responsiveness. Light signals are transmitted to GA and ABA-related genes via a phytochrome-interacting protein, PIL5, which functions as a transcription factor that directly or indirectly regulates the hormone-related genes. Recent papers have reported that the phytochrome-interacting proteins PIF3 and PIF4 physically interact with DELLA proteins in regulating gene expression during seedling growth (de Lucas et al. 2008; Feng et al. 2008). It has yet to be determined whether PIL5 also regulates seed germination through a similar mechanism, besides regulating transcription of *DELLA* genes. *SOM* is a direct target of PIL5 and regulates GA and ABA metabolic genes downstream of PIL5 via an as yet unknown mechanism. Elucidation of the molecular function of SOM is required to draw a whole picture of how light signals modulate hormone levels in seeds. There is evidence that the levels of GA and ABA in seeds are negatively regulated by each other. This regulatory loop would facilitate an efficient change in the ratio of the two antagonistic hormones in response to a signal. How one hormone regulates the levels of the other in this regulatory circuit requires further studies.

In the field condition, there are multiple environmental factors that change time to time and affect seed germination. Thus, plants must integrate different external cues to make a decision to germinate or not. Temperature is another critical factor that affects seed germination. High temperature inhibits germination (thermoinhibition), while exposure of imbibed seeds to cold temperature (stratification) stimulates germination in many plant species. Recent studies have shown that temperature modulates GA (and ABA) levels during thermoinhibition and stratification of Arabidopsis seeds through regulation of GA and ABA metabolic genes (Yamauchi et al. 2004; Toh et al. 2008). A PIL5-related bHLH protein, SPATULA (SPT), regulates GA biosynthetic genes in response to stratification (Penfield et al. 2005). Our improved understanding of light-induced seed germination and the identification of regulators of GA and ABA metabolic/signaling genes have started to allow us to study in further detail the mechanism by which multiple external cues are integrated to hormone metabolic/signaling pathways in seeds.

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