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REVIEW PAPER

Light perception and signalling by phytochrome A

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Abstract

In etiolated seedlings, phytochrome A (phyA) mediates very-low-fluence responses (VLFRs), which initiate de-etiolation at the interphase between the soil and above-ground environments, and high-irradiance responses (HIR), which complete de-etiolation under dense canopies and require more sustained activation with far-red light. Light-activated phyA is transported to the nucleus by FAR-RED ELONGATED HYPOCOTYL1 (FHY1). The nuclear pool of active phyA increases under prolonged far-red light of relatively high fluence rates. This condition maximizes the rate of FHY1–phyA complex assembly and disassembly, allowing FHY1 to return to the cytoplasm to translocate further phyA to the nucleus, to replace phyA degraded in the proteasome. The core signalling pathways downstream of nuclear phyA involve the negative regulation of CONSTITUTIVE PHOTOMORPHOGENIC 1, which targets for degradation transcription factors required for photomorphogenesis, and PHYTOCHROME-INTERACTING FACTORs, which are transcription factors that repress photomorphogenesis. Under sustained far-red light activation, released FHY1 can also be recruited with active phyA to target gene promoters as a transcriptional activator, and nuclear phyA signalling activates a positive regulatory loop involving BELL-LIKE HOMEODOMAIN 1 that reinforces the HIR.

Key words: CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), high-irradiance response (HIR), nuclear translocation, phytochrome, PHYTOCHROME INTERACTING FACTOR (PIF), very-low-fluence response (VLFR).

Introduction

Plant phytochromes are a family of red/far-red light photoreceptors that bear a linear tetrapyrrole chromophore attached through a cysteine residue to their N-terminal domain (Vierstra and Zhang, 2011). This review is focused on phytochrome A (phyA), a key member of the family with specific and shared functions. Phytochromes are synthesized in the inactive Pr form. Pr absorbs maximally in red light and, after excitation, relaxes into the active, Pfr form. In turn, Pfr has its maximum absorbance in far-red, which back-converts the molecule into Pr. Due to the partial overlap between Pr and Pfr absorption spectra (Fig. 1, inset), far-red light is able to transform a small proportion of the Pr molecules into Pfr. phyA monomers have a mol. wt of ~120 kDa and form phyA–phyA homodimers but no heterodimers with other family members (Sharrock and Clack, 2004).

Subcellular localization of phyA

In darkness, phyA is dispersed in the cytoplasm (Kircher *et al.*, 2002; Toledo-Ortiz *et al.*, 2010). There is nuclear phyB but no detectable nuclear phyA in dark-grown seedlings, and in chimeric phyA–phyB phytochromes this differential pattern is defined by the C-terminal domain (Oka *et al.*, 2012). Nuclear localization of phyA can be detected after 5 min of irradiation with red or far-red light that transform part of the Pr pool into Pfr (Toledo-Ortiz *et al.*, 2010). phyA lacks a known

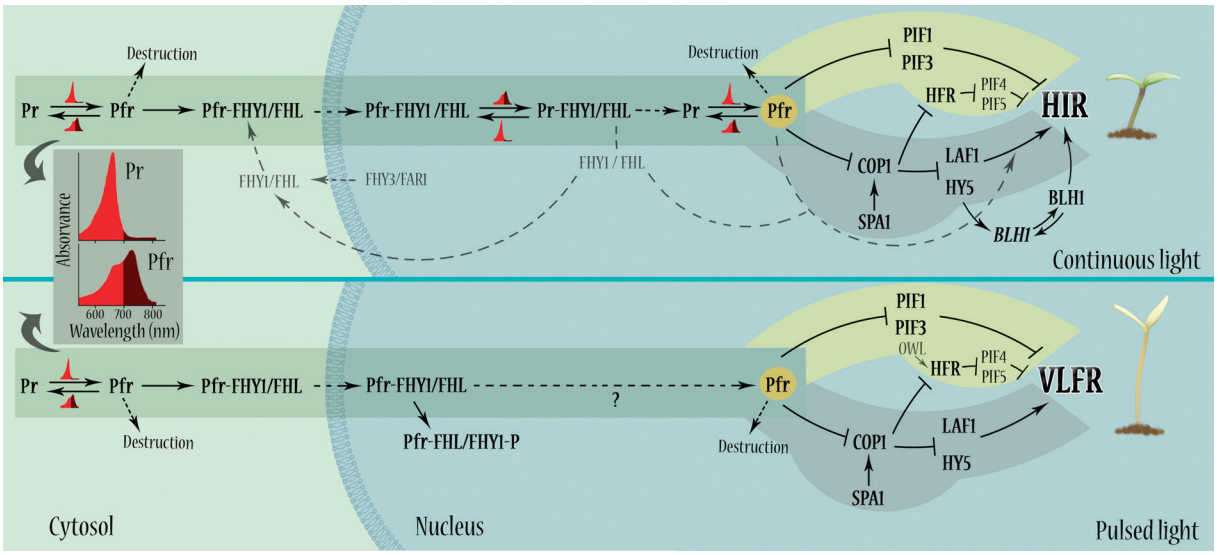

Fig. 1. Signalling by phyA under VLFR and HIR conditions. Three major modules are represented: the phyA perception module (Rausenberger *et al.*, 2011), the COP1 signalling pathway, and the PIF signalling module. Note that the HIR and VLFR differ in the perception module (Rausenberger *et al.*, 2011) and in the regulatory loops connected to the downstream signalling modules (Shen *et al.*, 2009; Staneloni *et al.*, 2009; Yang *et al.*, 2009; Chen *et al.*, 2012).

nuclear localization signal, and its nuclear presence depends primarily on FAR-RED ELONGATED HYPOCOTYL1 (FHY1) and secondarily on its homologue FHY1-LIKE (FHL) (Hiltbrunner *et al.*, 2005, 2006). The nuclear localization signal and the phyA-interaction domain of FHY1 (present in the N- and the C-terminus, respectively) are conserved in different species and are sufficient for FHY1 to transport phyA to the nucleus (Genoud *et al.*, 2008). Under daily photoperiods of far-red light, the number of nuclei with speckles containing phyA is higher during daytime than during the night, but the levels increase before the beginning of the day, arguing in favour of a circadian control of phyA localization (Kircher *et al.*, 2002).

phyA abundance

In dark-grown seedlings, phyA is the most abundant member of the phytochrome family (Sharrock and Clack, 2002). Light down-regulates the abundance of phyA at transcriptional and post-transcriptional levels.

Compared with full darkness, light perceived by phyA or phyB represses the expression of the *PHYA* gene (Quail, 1994; Cantón and Quail, 1999). This repression is accompanied by a rapid decrease in H3K4me3 and H3K9/14ac activating chromatin marks and a rapid increase in the H3K27me3 repressive mark at the *PHYA* promoter (Jang *et al.*, 2011). These chromatin modifications are mediated in part by phyB (Jang *et al.*, 2011).

While phyA is stable in the Pr form, the half-life of phyA Pfr is 0.5–2 h due to phyA ubiquitination and 26S proteasome degradation (Clough and Vierstra, 1997; Hennig *et al.*, 1999). Both, nuclear and cytoplasmic pools are degraded in the proteasome, but nuclear degradation is faster (Debrieux and Fankhauser, 2010; Toledo-Ortiz *et al.*, 2010). Degradation of phyA depends primarily on CULLIN1-based ubiquitin E3 ligases (Quint *et al.*, 2005; Debrieux *et al.*, 2013) and, under certain conditions, on CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1) ubiquitin E3 ligase (Seo *et al.*, 2004; Debrieux *et al.*, 2013). Under light/dark cycles, phyA accumulates during the night and becomes rapidly degraded during the day (Sharrock and Clack, 2002), despite the fact that *PHYA* promoter activity is maximal during the light phase due to the control by the circadian clock (Tóth *et al.*, 2001). phyA purified from dark-grown oat seedlings is phosphorylated (Lapko *et al.*, 1999), and purified recombinant oat phyA autophosphorylates (Han *et al.*, 2010). When expressed in *Arabidopsis*, mutations or deletions involving phosphorylation sites present at the N-terminal extension of phyA enhance the stability of oat phyA (Han *et al.*, 2010) but reduce the stability of *Arabidopsis* phyA (Trupkin *et al.*, 2007). These discrepancies might reflect the use of oat versus *Arabidopsis* phyA in the *Arabidopsis* background.

Pfr to Pr thermal reversion

Dark reversion is the light-independent conversion of Pfr into Pr and reveals the instability of Pfr likely when it is forming a heterodimer with Pr. This process affects the abundance of Pfr but not that of total phyA. In *Arabidopsis* only some accessions show thermal reversion of phyA Pfr, and the source of this natural variation is extragenic to *PHYA*, indicating that the cellular environment strongly affects Pfr stability (Hennig *et al.*, 1999; Eichhenberg *et al.*, 2000).

Molecular and cellular dynamics of phyA and the perception of light signals

The analysis of the relationship between light input and physiological outputs under controlled conditions has

traditionally defined three modes of photobiological action of phytochromes: the very-low-fluence responses (VLFRs), the low-fluence responses (LFRs), and the high-irradiance responses (HIRs) (Casal *et al.*, 1998). These photobiological modes of action partially reflect the contribution of different members of the phytochrome family to physiological responses.

The VLFR mediated by phyA

When imbibed seeds are exposed to a pulse of red light, the induction of germination above that of dark controls often shows a biphasic response to the fluence of the light pulse. The first phase, which typically saturates at 1 μmol. m^{-2} is the VLFR, and the second phase, observed at higher fluencies is the LFR (Cone *et al.*, 1985; Casal *et al.*, 1998). The VLFR is mediated by phyA and is therefore absent in the *phyA* mutant (Botto *et al.*, 1996; Shinomura *et al.*, 1996). The VLFR is triggered by the transformation of a small proportion of the phyA molecules into the Pfr form. All the phyA synthesized in a tissue not exposed to light is in the Pr form; if this tissue is exposed to far-red light, part of this Pr is going to be transformed into Pfr. Since only a small fraction of the phyA molecules needs to be in the Pfr form to cause a VLFR, this response mode can be initiated by far-red light and actually by any wavelength in the 300–780 nm range (Shinomura *et al.*, 1996).

The LFR involves the transformation of a large proportion of the Pr form into Pfr. It therefore requires higher red light fluencies and cannot be induced by far-red light. Actually, when given after red light, far-red light can cancel the LFR by reducing the level of Pfr established by red light to the much lower level established by far-red light. Thus, the LFR is reversible by far-red light but the VLFR is not, because the residual Pfr after far-red can be enough to saturate the VLFR. The LFR is mediated mainly by phyB and secondarily by other members of the phyB clade (Casal *et al.*, 1998). There are also some LFRs mediated by phyA (Stowe-Evans *et al.*, 2001); that is, there are specific cases where the signal transduction system is not saturated by the phyA Pfr levels established by far-red light. There are also intermediate signalling events where phyA effects show the features of an LFR (Shen *et al.*, 2009).

The HIR mediated by phyA

Seedlings grown in full darkness typically show fast axis (e.g. hypocotyl) extension growth, which becomes arrested upon exposure to light. The hypocotyl grows for several days at a rate that strongly depends on current light conditions, and therefore a brief light pulse often has no detectable effects on final hypocotyl length; continuous or prolonged exposure to light is required to inhibit growth significantly. Continuous red light or far-red light are very effective, acting via phyB and phyA, respectively (Quail *et al.*, 1995). Continuous red light can largely be replaced by hourly pulses of red light, and their effect is cancelled if immediately followed by far-red pulses, indicating that the action of continuous red light can be interpreted as a repeated LFR of phyB (Mazzella *et al.*, 1997). Only a very small proportion of the effect of continuous far-red can be replaced by hourly pulses of far-red (Mancinelli, 1994). When dark-grown seedlings are exposed to pulses of far-red light of different frequencies, two phases of response can be distinguished (Casal *et al.*, 2000; Zhou *et al.*, 2002). The first phase saturates with a frequency of one pulse every 120 min and corresponds to the contribution of the VLFR. The second phase is observed at frequencies above one pulse every 30 min, and that represents the specific contribution of the HIR.

VLFRs and HIRs can be genetically dissected at the level of the phyA molecule itself. The *phyA-302* allele, in which Glu777 (a residue conserved in angiosperm phytochromes) changed to lysine in the PAS2 motif of the C-terminal domain, retains VLFRs but lacks HIRs (Yanovsky *et al.*, 2002). This mutant fails to form phyA nuclear speckles, and has slightly reduced phyA levels in darkness, compensated by enhanced phyA stability under far-red light. Fusion proteins bearing the N-terminal domain of oat phyA, β-glucuronidase, green fluorescent protein, and a nuclear localization signal expressed in transgenic *Arabidopsis* show physiological activity in darkness and mediate VLFRs but not HIRs (Mateos *et al.*, 2006). Mutations at the serine-rich N-terminal domain of phyA can differentially affect VLFRs and HIRs (Casal *et al.*, 2002). The analysis of phyA–phyB chimeras indicates that the HIR observed under continuous far-red light requires *PHYA*-specific sequences primarily at the N-terminal domain (N-terminal extension, PAS, and PHY domains) and secondarily at the C-terminal domain (Oka *et al.*, 2012).

VLFRs can be induced by red or far-red light, but a higher fluence of the latter is required to reach saturation (Botto *et al.*, 1996; Shinomura *et al.*, 1996), which is consistent with the more efficient absorption of red than far-red light by Pr (Fig. 1). However, the HIR of phyA is stronger under far-red light than under red light, where much higher fluence rates are required (Franklin *et al.*, 2007). Why is far-red light more efficient than red light in the phyA HIR? HIRs show a much stronger dependency on fluence rate than repeated VLFRs caused by hourly far-red in the same system (Casal *et al.*, 2000). Why are HIRs so strongly fluence rate dependent?. These questions are beginning to be answered.

The most efficient wavelengths to cause HIRs are between the peaks of Pr and Pfr absorption. Long ago, the wavelength dependency of HIRs was modelled as a function of the rate of cycling between Pr and Pfr (Johnson, 1980; Shinomura *et al.*, 2000), and recent data indicate that cycling between Pr and Pfr is truly important for maximum phyA activity (Rausenberger *et al.*, 2011). The HIR involves primarily the nuclear pool of phyA (Genoud *et al.*, 2008; Toledo-Ortiz *et al.*, 2010). Starting from Pr in the cytoplasm, phototransformation yields Pfr, which binds to FHY1/FHL to migrate to the nucleus. In the nucleus, light is required to transform Pfr into Pr to release FHY1/FHL, otherwise the levels of cytoplasmic FHY1/FHL would limit continued transport of phyA to the nucleus and the build-up of the nuclear active phyA pool (Rausenberger *et al.*, 2011) (Fig. 1). Actually, the formation of speckles containing phyA shows the features of the

HIR as it is more intense under continuous than hourly far-red light (Casal *et al.*, 2002) and has a peak at 720 nm (Nagy and Schäfer, 2002). Then, light is needed again to transform the released Pr into active Pfr. In summary, why are HIRs strongly fluence rate dependent? High irradiances would enhance the accumulation of nuclear phyA Pfr by increasing the rate of FHY1/FHL–phyA complex assembly and disassembly (Rausenberger *et al.*, 2011). Why is far-red light more efficient than red light in the phyA HIR? The requirement for antagonistic photoconversion cycles to accumulate phyA Pfr in the nucleus would place the peak of effectiveness between the absorption peaks of Pr and Pfr (Rausenberger *et al.*, 2011). VLFRs saturate with very little Pfr and do not require high irradiances and antagonistic photoconversion cycles to build up the phyA Pfr nuclear pool (Fig. 1).

FAR-RED ELONGATED HYPOCOTYLS 3 (FHY3) and FAR-RED IMPAIRED RESPONSE 1 (FAR1) are proteins related to Mutator-like element transposases that directly activate the transcription of *FHY1* and *FHL* (Lin *et al.*, 2007). The *fhy3* (Whitelam *et al.*, 1993) and *far1* (Hudson *et al.*, 1999) mutants are impaired in HIRs but at least *fhy3* retains VLFRs (Yanovsky *et al.*, 2000). This suggests that the residual levels of *FHY1*/*FHL* in *fhy3* are sufficient for phyA translocation to the nucleus under VLFRs but limit the continued nuclear translocation of phyA required for HIRs.

The occurrence of phyA Pfr destruction is predicted to be essential for the wavelength and irradiance dependency of HIRs by generating the requirement for a continued flux of phyA towards the nucleus in order to supply new nuclear phyA Pfr (Rausenberger *et al.*, 2011). In support of this view, although *Physcomitrella patens* lacks phyA (the divergence of cryptogams of seed plants preceded the evolution of phyA), this cryptogam bears a phytochrome (Pp-PHY1) rapidly degraded in the Pfr form and transported to the nucleus in an FHY1-dependent manner, and exhibits HIRs under far-red light (Possart and Hiltbrunner, 2013). However, phyA fused to a nuclear localization signal is constitutively nuclear localized and its effects still retain features of the HIR, indicating that additional signalling modules must contribute to HIRs (Genoud *et al.*, 2008).

Signal transduction downstream of phyA

Although direct partners of phyA have been identified, the mechanism involved in the primary action of phyA on these targets has not been established. One of the ideas is that phytochromes could be light-regulated kinases (Yeh and Lagarias, 1998). For an updated discussion on the arguments in favour of and against this hypothesis, see Li *et al.* (2011).

The COP1 pathway

In dark-grown seedlings the complex formed by the ring finger E3 ligase COP1 and SUPPRESSOR OF PHYA-105 (SPA) is involved in the ubiquitination and targeting to subsequent degradation of several positive regulators of photomorphogenesis, including the basic leucine zipper transcription factor ELONGATED HYPOCOTYL 5 (HY5) (Saijo *et al.*, 2003), the atypical basic helix–loop–helix (bHLH) factor LONG HYPOCOTYL IN FAR-RED (HFR1) (Yang *et al.*, 2005), and the Myb transcription factor LONG AFTER FAR-RED LIGHT (LAF1) (Seo *et al.*, 2003). COP1 interacts directly with phyA, phyB, cryptochrome 1 (cry1), and cry2 (Lau and Deng, 2012). The association between COP1 and at least a pool of phyA (underphosphorylated phyA) is stronger in the absence of FHY1 (Saijo *et al.*, 2008), and for this reason we connect phyA with COP1 after phyA is released from FHY1 (Fig. 1). In response to light perceived by phytochromes or cryptochromes, COP1 migrates from the nucleus to the cytosol (Osterlund and Deng, 1998). There are additional, more rapid mechanisms of COP1 inactivation but they have not been identified in the case of phyA (Lau and Deng, 2012). This reduction in COP1 activity allows the recovery of the pools of HY5, LAF1, and HFR1, and the transition to photomorphogenesis (Saijo *et al.*, 2003; Seo *et al.*, 2003; Yang *et al.*, 2005). While HY5 is required for responses mediated by phyA, phyB, and cryptochrome, HFR1 is involved in phyA and cryptochrome signalling (Duek and Fankhauser, 2003) and LAF1 is involved in phyA signalling (Ballesteros *et al.*, 2001), suggesting that, in addition to COP1 inactivation, the contribution of HFR1 and LAF1 requires photoreceptor-specific events. At least some of these factors (e.g. HY5) operate in both VLFRs and HIRs (Crepy *et al.*, 2007) (Fig. 1). HFR1, LAF1, and HY5 are able to bind each other and at least the HFR1–LAF1 interaction reduces the ubiquitination and degradation of each other, but the physiological output does not show obvious interaction as the *hfr1*, *laf1*, and *hy5* mutants have largely additive phenotypes under continuous far-red light (Jang *et al.*, 2007, 2013).

The PHYTOCHROME-INTERACTING FACTOR (PIF) pathway

PIFs are a family of bHLH transcription factors that repress photomorphogenesis in darkness (Leivar and Quail, 2011; Jeong and Choi, 2013). Phytochromes bind PIFs, inducing their phosphorylation and reducing their activity by lowering their ability to bind DNA and/or causing their subsequent ubiquitination and degradation in the proteasome. Thus, phytochromes release the repression of photomorphogenesis imposed by PIFs. phyA binds to and causes the degradation of PIF1 (Shen *et al.*, 2005, 2008) and PIF3 (Bauer *et al.*, 2004), and therefore this is one of the pathways by which phyA promotes light responses under far-red, red, or blue light (Fig. 1). PIF3 co-localizes with phyA in early transient nuclear bodies (Bauer *et al.*, 2004). The normal degradation of phyA, PIF1, and PIF3 requires the nuclear- and plastid-localized protein HEMERA (HMR) (Chen *et al.*, 2010). PIF4 and PIF5 are targets of phyB, not of phyA, but phyA affects PIF4 and PIF5 indirectly because phyA enhances the abundance of HFR1, which binds PIF4 and PIF5 (Lorrain *et al.*, 2009), forming non-DNA-binding heterodimers (Hornitschek *et al.*, 2009).

Co-activation of target gene expression by FHY1/FHL

In the nucleus, the FHY1/FHL–phyA complex is recruited to the promoter of target genes by direct interaction with transcription factors such as HY5, PIF3, HFR1, and LAF1, where they co-activate transcription (Yang *et al.*, 2009) (Fig. 1). This mechanism of control of gene expression operates under HIR conditions, but probably not under VLFR conditions. Under VLFR conditions, phyA bound to FHY1/FHL remains in the Pfr form and this causes the rapid phosphorylation of a proportion of the pool of FHY1 (not of FHL), and phosphorylated FHY1 is unable to co-activate target gene expression (Shen *et al.*, 2009; Chen *et al.*, 2012).

Negative regulation of phyA signalling

EMPFINDLICHER IM DUNKELROTEN LICHT 1 (EID1) is an F-box protein predicted to target positive players in phyA signal transduction to ubiquitin-dependent proteolysis (Dieterle *et al.*, 2001). Under continuous light, the *eid1* mutation shows an exaggerated response and curiously shifts the most efficient wavelength of phyA activity from far-red to red light (Dieterle *et al.*, 2001; Zhou *et al.*, 2002). EID1 is localized diffusely within the nucleus of dark-grown seedlings and forms nuclear speckles under continuous far-red light (Marrocco *et al.*, 2006). The *spa1* mutant also shows enhanced sensitivity to light in both VLFRs and HIRs (Hoecker *et al.*, 1999; Baumgardt *et al.*, 2002), suggesting that it helps to enhance the activity of the residual COP1 in the light. Other members of the SPA family are also negative regulators of phyA-mediated de-etiolation (Laubinger and Hoecker, 2003). Although both SPA1 and EID1 are negative regulators of phyA signalling, the former has larger effects on VLFRs and the latter on HIRs (Zhou *et al.*, 2002).

HIR-specific regulatory loop

The expression of a given target gene may exhibit both the VLFR and HIR of phyA. Substitutions of the TGGA motif conserved in light-harvesting chlorophyll-binding genes of photosystem II (*Lhcb*) genes eliminate the HIR, leaving the VLFR component intact (Staneloni *et al.*, 2009). The BELL-LIKE HOMEODOMAIN 1 (BLH1) transcription factor, which belongs to the three-amino acid loop extension (TALE) superclass of homeobox genes, binds this motif (Staneloni *et al.*, 2009). The *blh1* mutants show a reduced HIR and normal VLFR not only for the expression of *Lhcb1*2* but also for hypocotyl growth and cotyledon unfolding. Mutations at the *BLH5* or *BLH6* genes also reduce HIRs (Staneloni *et al.*, 2009). A promoter substitution that enhances BLH1 binding to DNA shows a hyper-HIR.

The specificity of BLH1 action on HIR could be accounted for by the requirement to overcome a minimum threshold of BLH1 activity. In support of this view, the expression of *BLH1* is itself strongly dependent on far-red light fluence rate and, when *BLH1* is overexpressed, the VLFR is enhanced (Staneloni *et al.*, 2009). In wild-type plants, *BLH1* expression would not reach the minimum threshold under VLFR conditions. The expression of *BLH1* is controlled by BLH1 itself, suggesting that a positive feedback loop could help to overcome the threshold.

VLFR-specific signal transduction

Several proteins affect the VLFR and not the HIR. The *orientation under very low fluences of light 1* (*owl1*) mutant is impaired in VLFRs of seed germination and of seedling de-etiolation, but it retains apparently normal HIRs (Kneissl *et al.*, 2009). OWL1 is a ubiquitous J-domain protein that interacts in the nucleus with HFR1.

Other proteins include the nuclear protein GIGANTEA, which promotes VLFRs independently of its action on the circadian clock (Oliverio *et al.*, 2007). The cytoplasmic proteins PHYTOCHROME KINASE SUBSTRATE 1 (PKS1) and PKS2 form a regulatory loop that provides homeostasis to phyA signalling in the VLFR (Lariguet *et al.*, 2003). The DIMINUTO/DWARF 1/ENHANCED VERY-LOW FLUENCE RESPONSE 1 and DEETIOLATED 2 proteins involved in brassinosteroid synthesis reduce VLFRs and actually promote HIRs (Luccioni *et al.*, 2002). There is also substantial natural variation in VLFRs, which are weak in ecotypes Columbia (Yanovsky *et al.*, 1997), Nossen (Alconada-Magliano *et al.*, 2005), and Cape Verde Islands (Cvi) (Botto *et al.*, 2003) compared with Landsberg *erecta* (L*er*) (the quantitative trait loci that explain these polymorphism with L*er* are different). The *CRY2*-Cvi is a gain-of-function allele (compared with *CRY2*-L*er*) that enhances the VLFR of phyA (Botto *et al.*, 2003).

Cytoplasmic signalling

The *fhy1 fhl* double mutant lacks detectable nuclear phyA but retains phyA-mediated effects on gravitropism and phototropism, suggesting a role for cytoplasmic phyA (Rösler *et al.*, 2007). Actually, phyA and phototropin are able to interact physically at the plasma membrane (Jaedicke *et al.*, 2012). However, nuclear phyA accelerates the phototropic response, indicating that nuclear phyA is more effective than cytoplasmic phyA, and a low level of nuclear phyA signalling (not necessarily phyA itself) is still present in *fhy1 fhl* (Kami *et al.*, 2012).

Light signal perception by phyA in the natural environment

Perception of brief light exposures experienced by buried seeds

After dispersal, seeds may become buried. Prolonged burial in the darkness of the soil generates extreme sensitivity to light, and subsequent soil disturbance may transiently expose buried seeds to light (Scopel *et al.*, 1991; Botto, 1998). These seeds become induced to germinate. This strategy can be very effective for weeds of agricultural fields, which can be buried by early cultural practices and then briefly exposed

to light during soil disturbance for the immediate preparation of crop sowing, when the weed seeds receive information about the elimination of adult competitors by cultural practices. These brief light exposures are perceived by phyA (Botto *et al.*, 1996; Shinomura *et al.*, 1996). The acquisition of extreme light sensitivity during burial would involve synthesis of phyA (Shinomura *et al.*, 1996). This perception function corresponds to the VLFR. In *Arabidopsis* seeds, phyA controls the expression of 11% of the genome, including that of auxin- and gibberellin-related genes under VLFR conditions (Ibarra *et al.*, 2013). In some species (e.g. tomato, but not *Arabidopsis*), prolonged far-red light inhibits seed germination, indicating opposite effects of VLFRs and HIRs (Shichijo *et al.*, 2001). The HIR could contribute to prevent germination of seeds of these species under dense canopies.

Perception of light compared with darkness when organs are respectively above or below the surface of the soil

After germination of buried seeds, the shoot of a seedling may grow in darkness before reaching the light, which initiates the transition between the developmental pattern observed during heterotrophic growth in darkness (skotomorphogenesis) and photomorphogenesis. This transition, also called de-etiolation, involves a severe reduction of the rate of growth of the organ carrying the apical meristem to the surface (e.g. the hypocotyl), the expansion of the foliage (e.g. the cotyledons), the full development of the photosynthetic apparatus, and the expression of photosynthetic pigments.

Once a seedling emerges from the soil, it becomes exposed to light, but irradiance can still be weak due to the presence of litter or thatch (organic debris accumulated on the soil surface) (Fig. 2). The VLFR signalling mode of phyA would be important to initiate some steps of de-etiolation rapidly during this environmental transition. The early (e.g. 1 h) changes in gene expression taking place during de-etiolation are largely mediated by phyA under red (Tepperman *et al.*, 2006) or far-red light (Tepperman *et al.*, 2001). Many of these genes are transcription factors predicted to initiate the signalling network leading to de-etiolation. However, other processes, such as the hypocotyl growth to push the cotyledons out of the litter, should not respond strongly via the VLFR mode and actually the contribution of the VLFR to these processes is relatively weak when compared with the HIR or LFR (Yanovsky *et al.*, 1997). The case is different in grass seedlings where stem (mesocotyl) growth is fully arrested by a VLFR (Mandoli and Briggs, 1981) to place the meristem at soil level in order to avoid its mechanical damage.

In addition to its role under weak irradiances, phyA is crucial for de-etiolation under very dense canopies, where photosynthetic pigments severely absorb red and blue light, yielding an environment dominated by far-red light (Yanovsky *et al.*, 1995) (Fig. 2). Under these conditions, the *phyA* mutant shows a deficient transition between skoto- and photomorphogenesis and a high risk of lethality. The photosensory domain of phyA underwent adaptive evolution early in the history of flowering plants, suggesting a critical role in adaptation to deep shade (Mathews *et al.*, 2003).

The differences in the mechanisms involved in the VLFR and HIR (Fig. 1) are predicted to have evolved to fulfil the dual function of phyA, under conditions where no other photoreceptor is effective: very weak light and a low red/far-red light ratio (Fig. 2). Extreme light sensitivity (VLFR) is useful to initiate de-etiolation. The higher light threshold of the HIR (which requires higher irradiance and duration) would ensure that selected components of de-etiolation are not completed before the seedling has overtopped the litter. If the debris is overtopped in open areas (high red light, high blue light), phyB and cryptochromes become involved in completing de-etiolation. The versatile function of phyA is also evident in grass seedlings, where the promotion of coleoptile growth is

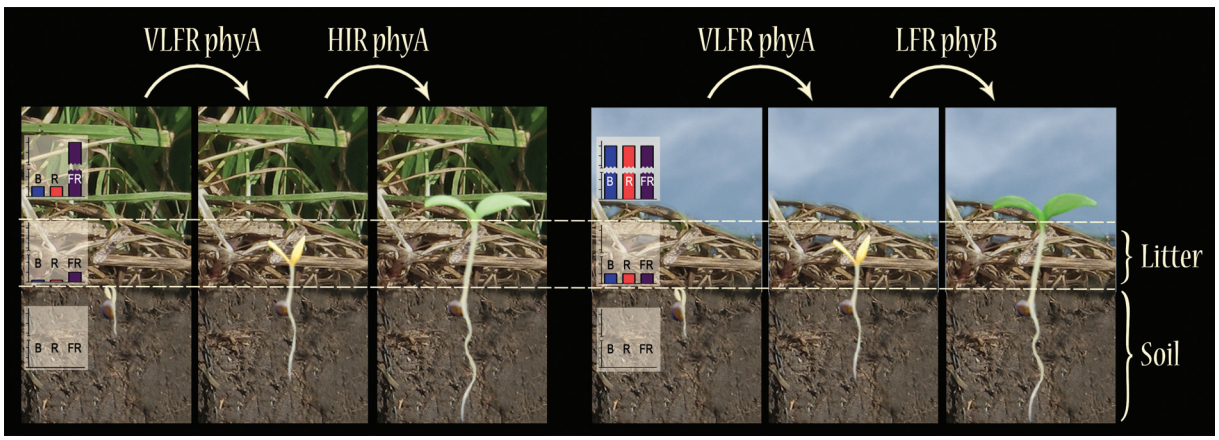

Fig. 2. Functional significance of phyA VLFR and phyA HIR during de-etiolation. Before emergence from the soil, the seedling is in full darkness. Upon emergence, the presence of litter can severely affect light penetration, and the high-sensitivity, VLFR pathway of phyA is required to initiate the first steps of de-etiolation. Once the layers of litter are overtopped, the seedling can experience high levels of red light (R), far-red light (FR), and blue light (B), and de-etiolation is completed by phyB (right boxes), which can operate synergistically with cryptochromes (Sellaro *et al.*, 2009). However, if after the layers of litter the seedling is shaded by deep canopy (left boxes), de-etiolation requires the HIR of phyA (Yanovsky *et al.*, 1995).

a VLFR but the synthesis of anthocyanin is a HIR (Casal *et al.*, 1996). The coleoptile has to grow once the mesocotyl has ceased expansion due to a VLFR but anthocyanin is useful to protect tissues against high irradiances. Therefore, these responses should occur at different light input levels.

Perception of the degree of shade

De-etiolation has to proceed under shade as well as in open places. However, beyond de-etiolation, the light signals caused by the presence of neighbours help plants to adjust their growth and physiology by a combination of changes that tend to reduce the degree of shade (i.e. the so-called shade-avoidance responses) and changes that help to acclimate the plant to the limitations of photosynthetic light caused by shade (Casal, 2013).

Shade is characterized by low irradiance and low red/far-red ratios because red light is absorbed by photosynthetic pigments while far-red is more efficiently transmitted and reflected. Therefore, shade imposes a conflicting signal to phyA because phyA activity (HIR) is favoured by increasing irradiances and by a low red/far-red ratio (Fig. 3A). Figure 3B illustrates this scenario. Seedlings of *Arabidopsis thaliana* were de-etiolated for 3 d and then transferred to the understorey of ryegrass canopies causing different degrees of shade. The length of the hypocotyl is plotted against the red/far-red ratio but irradiance also decreased with the red/far-red ratio. The *phyA phyB cry1 cry2* quadruple mutant showed no significant response to increasing shade. The *phyA cry1 cry2* mutant shows the response mediated by phyB that, as expected, increased with the red/far-red ratio. The *phyB cry1 cry2* mutant shows the response mediated by phyA and reveals the conflicting signal generated by shade. Deep shade is very effective to inhibit hypocotyl growth via phyA because it provides a low red/far-red ratio, despite the reduced irradiance. Intermediate degrees of shade are the least effective and, at the lowest degrees of shade (higher red far-red ratios), the contribution of phyA increases again because irradiance increases. Red/far-red ratios between 1.1 (unfiltered sunlight) and 0.3 are equally effective for phyA activity (Sellaro *et al.*, 2010), and this is consistent with the observation that under controlled conditions phyA activity is increased only by red plus far-red mixtures strongly enriched in far-red (Smith *et al.*, 1997). The contribution of phyA to the inhibition of hypocotyl growth in canopies with a red/far-red ratio >0.3 can successfully be modelled by using irradiance and not light quality as an input (Sellaro *et al.*, 2010). In de-etiolated tomato, shade by neighbours also releases stem growth from the inhibition imposed by phyA (Casal, 2013).

The *cry1 cry2* double mutant shows the combined contribution of phyA and phyB (Fig. 3B). It is obvious that both photoreceptors acting together do less than the sum of their individual contributions. The phyA-mediated inhibition at the lowest red/far-red ratios is less intense in the presence of phyB. The negative slope of hypocotyl length against the red/far-red ratio is not increased by phyA in the presence of phyB. phyA reduces the response to phyB signalling (Mazzella *et al.*, 1997; Cerdán *et al.*, 1999; Torres-Galea *et al.*, 2013). This is important because it renders the seedlings more sensitive to shade signals, particularly the early warning signal provided by the small reduction in the red/far-red ratio caused by neighbours that reflect far-red but do not shade (Casal, 1996). In turn, phyB interferes with phyA signalling (Hennig *et al.*, 2001; Zheng *et al.*, 2013).

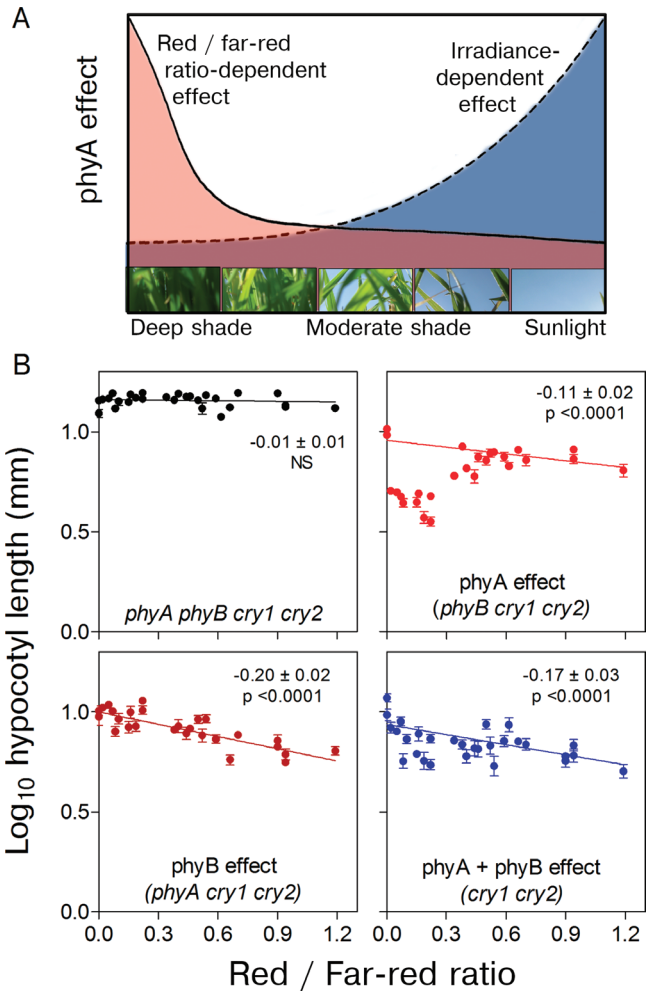

Fig. 3. Contrasting effects of canopy shade on the activity of phyA. (A) The absence of canopy shade favours phyA activity due to the presence of higher irradiances, and deep canopy shade favours phyA activity due to the presence of very low red/far-red ratios. (B) Contribution of phyA and phyB to the inhibition of hypocotyl growth as a function of the red/far-red ratio provided by ryegrass canopies of different density. The seedlings were de-etiolated for 3 d and then transferred to the different canopies for 4 d (see Supplementary Materials and methods available at *JXB* online). Each data point is the mean and SE of 10 seedlings. The slope ±SE of the regression lines is indicated. For *phyB cry1 cry2* and *cry1 cry2*, data for red/far-red ratios >0 and <0.5 were not included in the calculation of regression lines.

Perception of daylength

Many species adjust developmental transitions such as flowering time to the favourable season by perceiving the photoperiod. In *Arabidopsis*, phyA is one of the photoreceptors

involved in the perception of the difference between long and short days, particularly when daylength extensions contain far-red light (Johnson *et al.*, 1994). Under long days, phyA increases the stability of the CONSTANS protein (Valverde *et al.*, 2004), leading to enhanced expression of *FLOWERING LOCUS T* (Yanovsky and Kay, 2002), which promotes flowering. In rice (*Oryza sativa*), phyA is required to promote the expression of the flowering repressor gene *GRAIN NUMBER, PLANT HEIGHT AND HEADING DATE 7*, and to reduce the activity of the flowering inducer EARLY HEADING DATE 1 under long days (Osugi *et al.*, 2011). Soybean [*Glycine max* (L.) Merrill], is a short-day plant, but cultivars adapted to high latitudes are insensitive to photoperiod. Two of the loci that contribute to this insensitivity (so-called E3 and E4) are dysfunctional alleles of *PHYA* (Liu *et al.*, 2008; Watanabe *et al.*, 2009).

Conclusions

In order to adjust to the prevailing environment, plants require the ability to perceive the differences in light conditions and selectively modify the growth and developmental processes that require adjustment, while maintaining the homeostasis of those that should not be affected. The photoreceptor network has to be versatile in terms of light perception and downstream signal connectivity. phyA serves this purpose by perceiving the difference between full darkness and conditions where light is not enough to stimulate other photoreceptors (brief transient exposure to light, shade by litter, shade by dense canopies). Furthermore, phyA can distinguish between light signals that other photoreceptors are unable to perceive and generate discrete physiological outputs (VLFRs and HIRs). These selective features of phyA are based on the same chromophore used by other phytochromes and shared core signalling but phyA-specific nuclear translocation mechanisms and phyA-specific regulatory signalling loops decorating the core pathways.

Supplementary data

Supplementary data are available at *JXB* online.
Supplementary Materials and methods.

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