REVIEW

Phytochrome A in plants comprises two structurally and functionally distinct populations — water-soluble phyA′ and amphiphilic phyA″

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Abstract

Photoreceptor phytochrome A (phyA) plays a key role both in the individual development and in the evolution of higher plants. It acts in three distinct modes — far-red light-induced very low fluence responses (VLFRs), high irradiance responses (HIRs), and red/far-red–reversible low fluence responses (LFRs). Signal transduction from phyA includes its transportation from the cytoplasm into the nucleus and activation of light-responsive genes there. It is also active in the cytoplasm. Two types of phyA speckles were detected upon its light-induced nucleocytoplasmic partitioning and a fraction remained in the cytoplasm. In this review, we present a concept that this complex picture of the phyA action is due, at least partially, to the existence of two phyA types in the cell differing by the structure of the N-terminus, probably, by its serine phosphorylation. These are phosphorylated water-soluble phyA′ and underphosphorylated ambiquitous phyA″ represented by two fractions — water-soluble and membrane-associated. From the analysis of the phyA pools' activity in the regulation of phyA synthesis, seed germination, seedling establishment, and (proto)chlorophyll biosynthesis it is concluded that phyA″ is responsible for the regulation of seed germination, whereas in seedlings phyA′ mediates the VLFRs, and the water-soluble phyA″ fraction, the HIRs. The membrane-associated phyA″ is likely to be active in cytoplasmic photoregulatory events. Functional interaction between phyA and the defense-related hormone jasmonic acid is also considered.

Keywords Germination · Photomorphogenesis · Photoreaction · Phytochrome A · Polymorphism

Introduction: an emerging theme — diverse modes of phytochrome A action and its polymorphism

Investigation of the photoreceptor phytochrome occupies one of the central positions in contemporary plant photobiology due to its key role both in the individual processes of growth and development and in their evolution in general (Mathews et al. 2003; Casal 2013; Li et al. 2015). It is a biliprotein whose action is based on the photoisomerization of the chromophore that is induced most effectively by red light (R) and is reversed by far-red light (FR) (Braslavsky et al. 1997; Song et al. 1997). This reaction converts the initial physiologically inactive red-absorbing form (Pr), which is synthesized in the cytoplasm, into the active far-red–absorbing form (Pfr). The appearance of the latter is perceived by a plant as an informational signal that, after several steps of intracellular transduction, is realized in the modulation of a light-inducible genes' activity, and, as a result, in the plant's transition from the dark development (scotomorphogenesis) to the light development (photomorphogenesis) (Casal et al. 2014).

Light signal transduction by phytochrome can be briefly presented as follows: photoreceptor in the Pfr form is translocated from the cytoplasm into the nucleus (Klose et al. 2015), where it participates in the degradation of the phytochrome interacting factors blocking photomorphogenesis and activates the positive photomorphogenic factors. The latter induces transcriptional activity of the photoresponsive genes and initiation of the photomorphogenic mode of plant development (Sheerin and Hiltbrunner 2017; Lee and Choi 2017). Functional activity of phytochrome in the cytoplasm characterized by fast (seconds, minutes) biophysical and biochemical responses is also found (Hughes 2013).

The character of the photophysiological response mediated by phytochrome is determined by the photoreversibility of the Pr → Pfr photoconversion and by the spectral composition of the actinic light. Indeed, many plant photoresponses reveal themselves under R (induction of seed germination, stem elongation inhibition, cotyledon opening) and its effect can be abolished by FR. However, there are light responses that do not fit into this classical scheme. A key feature distinguishing them from the "classical" ones is their inability to be reverted by FR. On the contrary, the latter activates them with relatively high effectiveness. Detailed investigation of their light fluence and spectral dependences revealed three photoresponse modes — besides the photoreversible low fluence responses (LFRs), there exist the FR-induced nonphotoreversible very low fluence responses (VLFRs) and high irradiance responses (HIRs) (Casal et al. 1998).

Along with the detection of the various photoresponse modes, the heterogeneity of the photoreceptor itself was discovered — the existence of its species differing by light lability (Brockman and Schäfer 1982), immunochemical properties (Shimazaki and Pratt 1985), and spectral and photochemical characteristics (Sineshchekov and Sineshchekov 1987, 1989). A breakthrough was the discovery of a small family of phytochrome genes and their products (Sharrock and Quail 1989), for instance, five in *Arabidopsis* (Clack et al. 1994) and three in rice (Takano et al. 2005), two of which are the major ones both in content and functionality — light-labile phytochrome A (type I) in etiolated plants and light-stable phytochrome B (type II) in light-grown. Their existence could explain both the heterogeneity of the photoreceptor and the complex picture of its action. Using phytochrome-deficient mutants, it was shown that the LFRs are mediated by phyB, whereas the VLFRs and HIRs, by

phyA (Casal et al 1998). The latter two types are functionally different and operate through distinct pathways and/or in different cells (Yanovsky et al. 1997; Casal 2000).

In our group, it was found that the phytochrome system could be even more complex than that. Two types of phytochrome A were detected in mono- and dicotyledons differing by fluorescent and photochemical properties — the post-translationally modified products of the phytochrome A gene, phyA′ and phyA″, and it was shown that they are responsible for the two modes of the phyA photoresponses, the VLFRs and HIRs (see reviews Sineshchekov 1995a, 2010, 2019 and the original papers by the same author cited therein). In this review, we are exploring further this possibility considering their functional roles in the regulation of the key stages of plant development — induction of seed germination and seedling establishment.

The heterogeneity of phytochrome A: distinct molecular species and conformers

Investigations of phytochrome in situ are strongly hampered by its very low content in plant tissues, their high light scattering, and the presence of other pigments in them. The discoverers of phytochrome employed absorption difference spectroscopy, which for long was the only method of its determination in plant tissues (Butler et al. 1959). In our group, low-temperature (T) fluorescence of phytochrome was detected in etiolated plants (Fig. 1) and based on that a highly sensitive and informative method of its in vivo assay was developed (Fig. 2) (see reviews Sineshchekov 1995a, 2010, 2019). With its use, phytochrome in its Pr form was described by fluorescent and photochemical parameters and,

Fig. 1 Fluorescence emission ($\lambda_{ex} = 630$ nm) and excitation ($\lambda_{em} = 700$ nm) spectra of phytochrome in the Pr form in stems of etiolated pea seedlings at 77 K and 200 K. Excitation spectrum with the maximum at 375 nm was calculated in the region 350–500 nm with due consideration of green background fluorescence with an excitation maximum at 420 nm. (From Sineshchekov and Sineshchekov 1987, 1989)
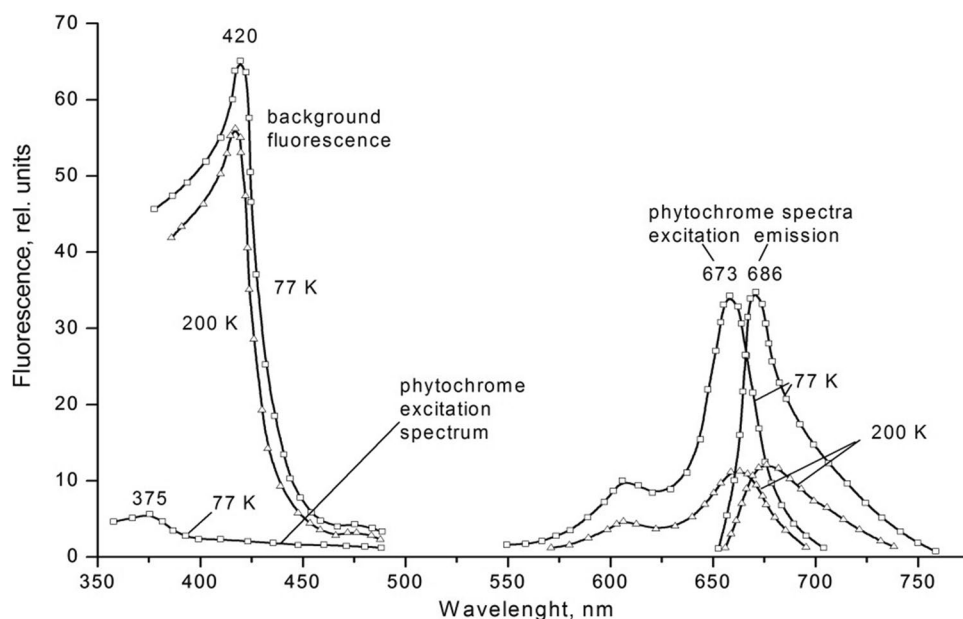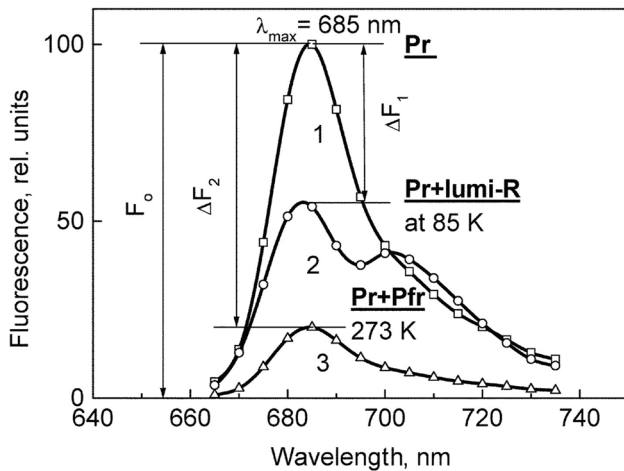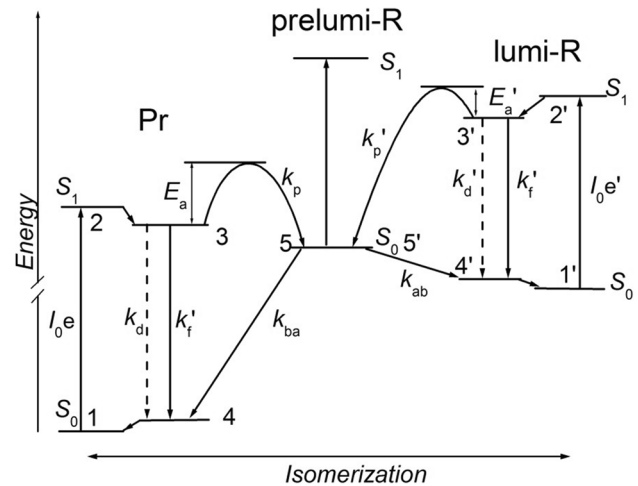
Fig. 2 Low-temperature (T) fluorescence emission spectra (λ_{ex} = 632.8 nm) of phytochrome in situ in its initial red light-absorbing Pr form and after its photochemical conversions at low T into lumi-R and at ambient T into the far-red–absorbing form Pfr: 1—etiolated (wheat) coleoptiles at 85 K, 2—the same sample after saturating red illumination (λ_a = 632.8 nm) (R) at 85 K partially converting Pr into lumi-R, the first stable at low T photoproduct, and 3—the same sample after thawing at 273 K and R illumination converting Pr into Pfr and freezing again at 85 K. Four major parameters can be obtained from these spectra: position of the spectrum (λ_{max}); phytochrome content ([P_{tot}] ≈ F_0); extent of the Pr conversion into lumi-R ($\gamma_1 = \Delta F_1/F_0$) and the far-red light-absorbing form Pfr ($\gamma_2 = \Delta F_2/F_0$). The maximum at 685 nm belongs to Pr and shoulder at 704 nm, to lumi-R; Pfr does not fluoresce even at low T. (From Sineshchekov 1994, 2004)

Fig. 3 The energy level scheme of the photoisomerization reaction of the initial red-absorbing phytochrome form (Pr) into the first photoproduct (lumi-R) stable at low temperatures via a short-lived unstable orthogonal intermediate (prelumi-R). At 85 K and saturating red illumination (λ_a = 632.8), there is a photoequilibrium between Pr and lumi-R determined by the rates of the forward (Pr → lumi-R) and reversed (lumi-R → Pfr) photoreactions (corresponds to spectrum 2 in Fig. 1). The activation barrier in the excited state, E_a for Pr and E_a' for lumi-R, determines the photochemical properties of Pr and lumi-R and the extent of the Pr photoconversion to reach a photoequilibrium with lumi-R at low T (γ_1 varies for different Pr species from 0 to 0.5). At ambient temperatures, this barrier is easily overcome and the extent of the Pr → Pfr photoconversion remains relatively constant, 0.75–0.85. (From Sineshchekov and Sineshchekov 1990; Sineshchekov 1994, 1995a)

proceeding from their temperature dependence, the initial photoisomerization reaction of Pr was characterized as an activation process with an energy barrier E_a in the excited state (Sineshchekov and Sineshchekov 1990; Sineshchekov 1994, 1995a) (Fig. 3). It reflects the degree of freedom of the *cis–trans* flip-flop in the Pr → lumi-R conversion in the chromophore pocket. At low T (77–85 K), the Pr → Pfr conversion is stopped at the stage of the photoproduct lumi-R. E_a of the Pr and lumi-R states determine the quantum yield of the direct (Pr → lumi-R) and reverse (lumi-R → Pr) photoreactions (Fig. 3) and, hence, the photoequilibrium between Pr and lumi-R at the wavelength of the actinic light. The Pr/lumi-R ratio in the photoequilibrium is measured as the extent of the Pr conversion into lumi-R under saturating R, γ_1 (Fig. 2).

Two phenomenological phytochrome types — photoactive at low T Pr′ (with γ_1' = 0.5) and inactive Pr″ (γ_1'' = 0) — were detected. With these individual γ_1 parameters, their content and proportion could be determined from experimental γ_1 values of a sample as described in (Sineshchekov 1994). Experiments with phytochrome mutants lacking phyB (Sineshchekov 1995b; Sineshchekov et al., 1998, 1999a, b, 2006) and heterologous transgenic systems expressing recombinant phyA (Sineshchekov et al.

2001a, 2013) have shown that both the types belong to phyA, whereas phyB is represented only by Pr″ (Sineshchekov et al. 2000). The two phyAs were characterized by physicochemical parameters and phenomenological properties (Table 1), their content and proportion being strongly dependent on plant species and organ/tissue used, plant age and stage of development, physiological conditions, and environmental factors (light, dehydration, water stress, etc.) (see Table 1 in (Sineshchekov 2019)). Along with the two phyA gene products, heterogeneity within phyA′ was also detected: it comprised several conformers differing by fluorescence and photochemical characteristics (Sineshchekov and Akhobadze 1992).

The heterogeneity is not limited to phyA: two species of the fern *Adiantum* phy1 (expressed in transgenic *Arabidopsis*) like the two phyAs were also distinguished (Sineshchekov et al. 2014a) suggesting that this differentiation preceded the evolution of the appearance of the higher plants. The existence of conformers like those of phyA′ was demonstrated in the case of the cyanobacterial phytochrome Cph1 (Sineshchekov et al. 2014b; Sineshchekov and Bekasova 2020). We can thus distinguish three levels of organization of the phytochrome system: besides the products of phytochrome genes, there are another two levels

Table 1 Two native phytochrome A types in mono- and dicotyledonous plants

Parameter	Phytochrome type	
	phyA′	phyA″
Position of emission/absorption maxima, λ_{max}, nm	Longer wavelength (685/672)	Shorter wavelength (680/667)
Half-band width, nm	22–24	30
Extent of Pr→lumi-R conversion at 85 K, γ_1	0.49 ± 0.03	≤ 0.05
Activation barrier in excited state, E_a, kJ mol^{-1}	≤ 1	≥ 10
Extent of Pr→Pfr conversion at 273 K, γ_2	0.80–0.85	0.75
Light lability	Light labile	Relatively light stable
Hydrophilicity/hydrophobicity	Water–soluble	Ambiquitous—soluble and membrane-(protein–) associated
Content in etiolated tissues	Major, variable	Minor, saturated, conserved

— post-translationally modified species and conformers within one molecular type.

To the nature of the phytochrome A differentiation

Our experiments with phyA mutants have shown that the site(-s) responsible for the phyA′ formation is at the N-terminal extension (NTE) (Sineshchekov 1995a, b, 2010, 2019). It is likely a serine residue(s) within the 10 N-terminal serines (in rice phyA expressed in *Arabidopsis*) since their substitution by alanines (phyA SA) resulted in the presence of only phyA″ in the sample (lacking phyA′) (Sineshchekov et al. 2018). Taking into consideration that phytochrome A is a phosphoprotein autophosphorylated in the Pr state in darkness at serines 8 and 18 (in oats) (see review (Hoang et al. 2019)), we could assume that phyA′ formation is the result of phyA″ serine phosphorylation. However, this hypothetical serine(s) is not serines 8 and/ or 18 as it was demonstrated in the experiments on phyA mutants with the S/A substitution at positions 8 and 18 (of oat phyA expressed in transgenic *Arabidopsis*) (Sineshchekov et al. 2019).

The presumed phosphorylation of phyA at the NTE affects the conformation of the chromophore pocket and the photochemical properties of the pigment (E_a of the photoreaction) because of the appearance in the molecule of a charged phosphoryl group. Although the interaction of the NTE with the chromophore in the Pr form of phyA is much less pronounced than in the Pfr form (Song et al. 2018), its effect is seen in changes in the E_a of the Pr → lumi-R isomerization (see above). These changes are likely due to variations in the conformation of the chromophore pocket

and steric hindrances to the isomerization. The phyA modification with the formation of the presumably phosphorylated phyA′ from phyA″ may thus change the conformation of the pigment.

Another important outcome could be the changes in the hydrophobicity/hydrophilicity of the pigment. phyA is represented by both the phyA pools, phyA′, and phyA″, in the supernatant from etiolated maize coleoptiles (Sineshchekov et al. 1994) or *Arabidopsis* hypocotyls (V. Sineshchekov and M. Zeidler, unpublished results), whereas in the sediment, primarily or exclusively by phyA″ in agreement with the data on the existence of the membrane-(protein-) associated phyA fraction (see Lamparter et al. 1992; Terry et al. 1992). phyA″ thus shows amphiphilic properties and can be called an "ambiquitous phyA type," in analogy with the "ambiquitous enzymes" — having the ability to reversibly bind to subcellular structures (Clegg 1984). Also, deep dehydration of etiolated tissues brings about a disappearance of phyA′ (Sineshchekov 2006) in line with the notion that phyA′ is a hydrophilic protein.

In general, the existence of the two phyA pools so profoundly different in their properties allows an assumption that they perform essentially distinct functions in plants each mediating its "own" photoresponse type, VLFRs, or HIRs. Noteworthy in this connection is that the two phyA pools form different types of nuclear bodies after the phyA light-induced transfer from the cytoplasm (Sineshchekov et al. 2014c). Nagy et al. (2001) distinguished two pools of phyA in the Pfr form one of which makes distinct types of loci in the nucleus and the other is homogeneously distributed in the cytoplasm. It is tempting to connect these phenomena with the existence of the two phyA types — water-soluble phyA′ and phyA″ represented by two fractions, water-soluble and membrane-associated.

Light as a major factor determining the content and activity of the two phyA populations

In darkness, the phyA pools' balance is intimately connected with the kinase/phosphatase equilibrium in the cell. This is pointed at by the fact that the phosphatase inhibition by okadaic and cantharidic acids (Sineshchekov et al. 2013) and by NaF (Sineshchekov et al. 2021a, b) shifts the ratio towards phyA″ due to the accelerated destruction of the labile phyA′. On the other hand, phytochrome kinase substrates (PKS1 and PKS2), which are transphosphorylated by phyA (Lariguet et al. 2003), can contribute to the maintenance of a proper phyA′/phyA″ ratio (Sineshchekov and Fankhauser 2004). In the double *pks1pks2* (*Arabidopsis*) mutant, the equilibrium between phyA′ and phyA″ shifted considerably towards phyA″. The phyA′/phyA″ ratio is also affected by the cytosolic pH with the phyA′ proportion reaching a maximum at pH values close to the physiological (Sineshchekov et al. 2013).

The most efficient factor affecting the phytochrome A species is, however, light. Red light brings about a rapid (minutes) and selective destruction of the phyA′ form in etiolated tissues, whereas phyA″ remains relatively constant (Sineshchekov et al. 1999a, b; Sineshchekov and Weller 2004). This results in the shift of the phyA′/phyA″ ratio strongly towards phyA″ which correlates with the detection of the two phyA fractions differing by destruction kinetics (Clough and Vierstra 1997). From this, one may assume that relatively light-stable phyA″ is a dominating or the only phyA species in light-grown plants.

A more complex picture is observed upon the action of FR: depending on the plant species and illumination conditions there could be a decline of the total phyA content with or without violation of the phyA′/phyA″ content. In pea grown under constant FR (FRc), [phyA] dropped down considerably; however, the phyA pools' equilibrium remained essentially the same as in the control (Sineshchekov and Weller 2004). This effect is likely to relate to the inhibition by phyA of its synthesis (Cantón and Quail 1999; Clough et al. 1995). This notion is supported by the fact that in epicotyls of the pea mutant *lip*, which reveals the deetiolated phenotype in darkness, total phyA content drops down significantly without violation of the phyA′/phyA″ proportion, too (Sineshchekov et al. 1995). Interestingly, this effect of the mutation was lacking in roots, suggesting that the state of phyA in roots differs from that in epicotyls (for the specificity of the phyA state in roots, see also (Sineshchekov et al. 2021b)).
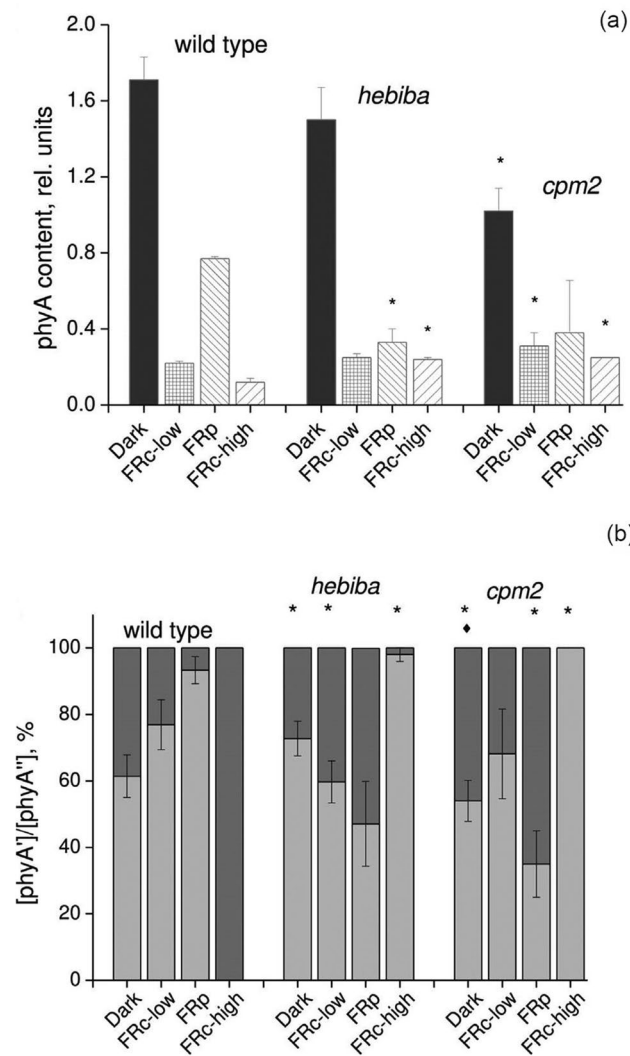
A strong [phyA] decline (ca 95%) was observed in the coleoptiles of FRc-grown wild-type (WT) rice (*Oryza sativa* L. cv. Nipponbare) and its *phyB* mutant (Sineshchekov et al. 2006) suggesting that phyB, as expected, is not involved in this process. (The phyA′/phyA″ proportion could not be measured because of low residual phyA.) The FRc-induced inhibition of [phyA] was also observed on another rice variety, *Oryza sativa* L. cv. Nihonmasari, followed by a shift in the phyA′/phyA″ proportion towards phyA″. FRc regimes differing approx. 50-fold by fluence rate (FRc-high and FRc-low) produced a similar inhibiting effect and pulsed FR (FRp) was ineffective (Sineshchekov et al. 2004b) or less effective (Sineshchekov et al. 2021a) than FRc (Fig. 4).

Experiments with rice mutants, *hebiba*, and *cpm2*, deficient in the defense-related phytohormone jasmonic acid (JA), which is engaged in plants' responses to biotic and abiotic stress, have shown that it is deeply involved in the FR-induced effects (Sineshchekov et al. 2004b, 2021a). The latter revealed a strong dependence on seedlings' genotype, their age, and illumination conditions. FRc (HIRs) was as inhibiting in the mutants as in the WT, whereas FRp (the VLFRs) was ineffective in *hebiba* or more effective than in the WT (Fig. 4). The decline in the phyA content upon FRc was followed by the domination of phyA′ in the mutants and of phyA″ in the WT suggesting that in the mutants there is a stimulation of the phyA″ → phyA′ conversion, which is slowed down by JA in the WT. Besides, in the mutants, the direction of the FRp effect (domination of phyA″ in the phyA′/phyA″ balance) was opposite to that of FRc (Fig. 4). The observed specificity of the JA influence on the FR effects in the WT can relate to JA participation in phyA destruction documented in (Sineshchekov et al. 2004b; Riemann et al. 2009). In the JA mutants, the light-induced destruction of phyA in its light-labile form phyA′ is slowed down, which brings about the domination of the latter in contrast to the WT.
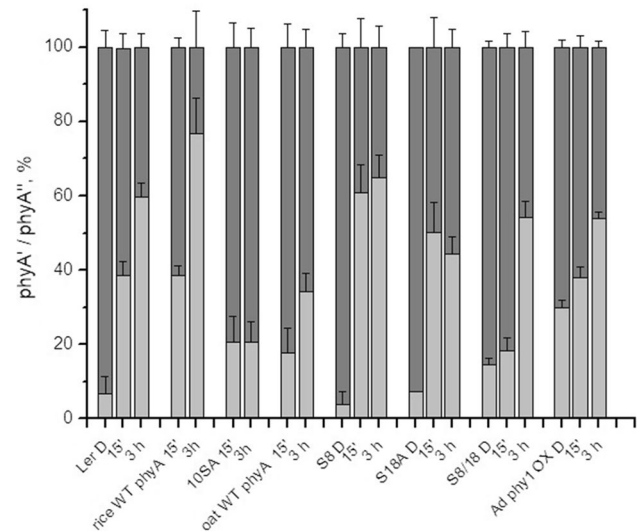
An unexpected light effect on phyA was observed after light induction of (*Arabidopsis*) seed germination. White light preillumination of seeds (tungsten lamp,15 min or 3 h) caused a considerable redistribution of the phyA pools in dark-grown seedlings towards phyA′ without violation of the total phyA content (Fig. 5), possibly, via promotion of the phyA″ phosphorylation (Sineshchekov et al. 2014a,c). This effect may relate to that observed by Magliano and Casal (2004) when R preillumination of *Arabidopsis* seeds induced an inhibitory effect on hypocotyl elongation in seedlings grown in the dark. On the other hand, it may result from the stimulation of seedling development because the total phyA content and the phyA′/phyA″ proportion increase with the seedling's age (Sineshchekov 1994; Sineshchekov et al. 2013).

A different light effect was seen in rice coleoptiles, WT and *phyB* mutant (Sineshchekov et al. 2006). The phyA content was higher in the *phyB* mutant, compared with WT. This might be the consequence of the inhibitory function of phyB

Fig. 5 Effect of germination-inducing seed preillumination on the relative content of the two phyA pools, phyA′ and phyA″ (light grey and dark grey, respectively), in the etiolated coleoptiles of wild-type (WT) *Arabidopsis* (Ler) and its transgenic lines deficient in endogenous phyA but expressing foreign phyA — (1) rice WT phyA (WT phyA) and mutant rice phyA with the substitution of 10 serines at the NTE for alanines (10SA), and (2) oat WT phyA and mutant oat phyA with the S8A, S18A, S8/18A substitutions. The *Arabidopsis* line expressing phy1 of fern *Adiantum capilus veneris* (Ad phy1 OX) was also used. Imbibed seeds were preilluminated with white light (W) for 15 min or 3 h to induce germination. Mean values with standard error (SE) are presented. (Modified from Sineshchekov et al. 2014a, b, c, 2018, 2019)

Fig. 4 The total content of phytochrome A in the coleoptiles of the wild-type seedlings of rice (*Oryza sativa* L. ssp. Japonica cv. Nihonmasari) and its *hebiba* and *cpm2* mutants deficient in the hormone jasmonic acid (JA) (**a**) and the proportion of its two native types, phyA′ (light grey) /phyA″ (grey) (**b**). The illumination conditions: far-red constant light FRc of high irradiance (FRc-high, $\lambda_a = 740$ nm, 0.2 μmol m^{-2} s^{-1}) (favorable for the HIRs), pulsed FR (FRp, 6 min light/54 min dark, 0.2 μmol m^{-2} s^{-1}) (favorable for the VLFRs) and constant FR of low irradiance (FRc-low, 0.004 μmol m^{-2} s.$^{-1}$), which may have the properties of the two response modes (HIR and VLFR). **a** The effects of the FR light of all the regimes on the phyA content are statistically significant for all the lines. The significant differences between the mutants and the WT are indicated by asterisks, and there is no difference between the mutants. **b** The statistically significant light effects on the phyA pools' ratio in all the lines are Dark vs. FRp and FRc-high, the statistically significant differences between the mutants and the WT are indicated by asterisks, and between the mutants, by the diamond symbol. (From Sineshchekov et al. 2021a)

in the dark-grown rice seedlings due to the illumination of seeds during their maturation. This is supported by the fact that the WT coleoptile is shorter than that of the *phyB* mutant (see below). The proportion of the two phyA pools

remains, however, the same in WT and the *phyB* mutant suggesting that the phyB influence is localized upstream of the posttranslational modification of phyA. Collectively, these different light-induced modifications of the phyA turnover — inhibition of the phyA synthesis, preferential phyA′ degradation, and promotion of the phyA″ conversion into phyA′ — result in the modulation of their photophysiological activity thus contributing to the fine-tuning of the photoreceptor apparatus.

## Functional role of phytochrome A in the regulation of seed germination

Phytochromes affect seed germination inducing or inhibiting it, with phyB and phyA playing a key role in these processes (Casal and Sánchez 1998; Franklin and Quail 2010). Seeds of some plant species can germinate both in light and in darkness, they have sufficient [Pfr] to induce germination (Takaki 2001). phyB regulates germination through detection of the R:FR ratio (LFR) including the conditions under the canopy shade of the deciduous forests (Kendrick 1976; Ballaré and Casal 2000). phyA promotes germination in the

VLFR and HIR modes under the conditions of the FR light domination including those of the evergreen forests (Botto et al. 1996; Johnson et al. 1994; Hennig et al. 2001; Seo et al. 2009; Takaki 2001).

In dry seeds, there is a domination of phyB with a very low level of phyA (Tobin and Briggs 1969; Hauser et al. 1997; Somers and Quail 1995). Pre-existing in dry *Arabidopsis* seeds phyB mediates the R-induced germination 3 h after the start of imbibition — the typical R/FR LFR (Shinomura et al. 1994; Botto et al. 1995). Germination under the regulation of phyA is observed only after its de novo synthesis 48 h after the onset of (*Arabidopsis)* seed imbibition (Shinomura et al. 1996).

In early works, it was shown that the "seed phytochrome" differs from the "seedling phytochrome" in immunological traits and stability (in *Avena sativa)* (Spruit and Mancinelli 1969; Hilton and Thomas 1985; Tokuhisa and Quail 1987). Our experiments suggest that the state of the phyA pools in developing embryos may also substantially differ from that in seedlings. In wheat embryos at the stage of their breaking through 15 h after the onset of imbibition, the proportion of phyA′/ phyA″ is approx. 50/50, whereas in coleoptiles 80/20 (Sineshchekov et al. 2001a). In imbibing seeds of pea and bean, we observed the active rise of [phyA] and the concomitant increase of the phyA′ proportion in seeds 8 h after the onset of the imbibition (Sineshchekov et al. 1998) that is much earlier than the onset of the phyA synthesis in *Arabidopsis* (Shinomura et al. 1996). The phyA′/phyA″ balance with phyA′ domination characteristic for the seedlings establishes after 28–30 h of imbibition (Sineshchekov et al. 1989). Of interest in this context is the effect of dehydration on phyA in etiolated tissues (Sineshchekov 2006). Even a considerable loss of water (up to 75–85% of the initial fresh weight of etiolated coleoptiles of barley and maize) does not bring about noticeable alterations in the total phyA content and the phyA′/phyA″ proportion pointing to relative stability of the phyA′/phyA″ system in this regard. However, extreme dehydration (loss of weight ≈90%) caused a transformation of phyA′ into phyA″, which was not reversed by rehydration. We may thus hypothesize that phyA in dry and imbibing seeds is represented by its phyA″ isoform and this form is active in the regulation of germination.

This assumption is supported by our experiments on transgenic *Arabidopsis* expressing rice phyA SA represented by phyA″ (Sineshchekov et al. 2018). It was found that the germination rate of these transgenic seeds after preillumination (W, 15 min and 3 h was approx. the same as that of the *Arabidopsis* line expressing WT rice phyA and the L*er* variety (Fig. 6). Earlier, Kneissl et al. (2008) have observed that germination of the PHYA SA-expressing lines was more efficient than the WT PHYA-expressing lines and this effect was stronger under R light suggesting that it can sense the R- and FR-light pulses. Collectively,
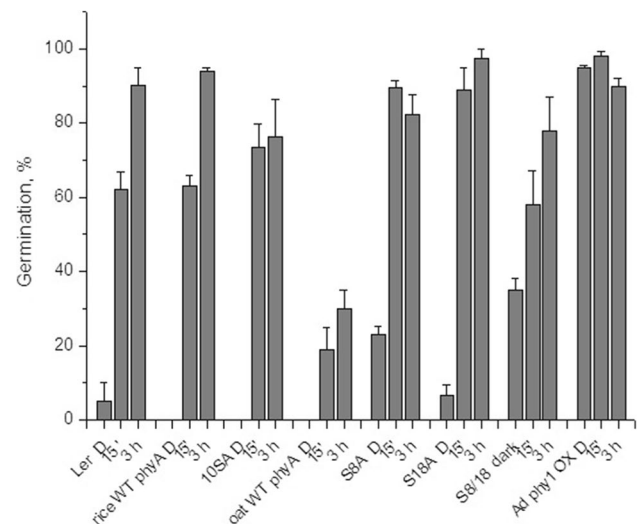
Fig. 6 White light-induced germination of the wild-type (WT) *Arabidopsis* and its transgenic lines expressing foreign WT and mutant phytochrome A. (For the description of the *Arabidopsis* lines used and measuring conditions, see the legend to Fig. 5). (Modified from Sineshchekov et al. 2014a, b, c, 2018, 2019)

these data allow a conclusion that phyA″ is active in the stimulation of seed germination under the conditions of HIR or LFR.

Phosphorylation of phyA at serines 8 and 18 (in oat phyA overexpressed in *Arabidopsis*) does not participate in the differentiation of the photoreceptor into the phyA′ and phyA″ pools (see above and (Sineshchekov et al. 2019)). However, it regulates the functional properties of the photoreceptor (Han et al. 2010). We investigated how this may affect the phyA″ activity in the germination induction. Transgenic *Arabidopsis* overexpressing WT *Avena sativa* phyA (AsAox) is characterized by a low germination rate — less than 30% even after 3 h white light (W) pre-illumination (Fig. 6). However, the transgenic *Arabidopsis* with the mutant oat phyA — S8A, S18A, S8/18A — germinated in darkness (up to 25%) and reached a germination rate of 90–98% even after 15-min preillumination. Thus, this block of phosphorylation by the SA substitution makes phyA″ more functionally effective supporting the notion by Han et al. (2010) that phosphorylation at these sites serves as an instrument of the regulation of phyA activity.

The role of phyA′ in the regulation of seed germination is not quite clear. Of interest in this respect is truncated oat Δ6-12 phyA because according to our data (Sineshchekov et al. 2014c), it is represented by phyA′. Trupkin et al. (2007) have documented that the seeds of *Arabidopsis* expressing truncated *Arabidopsis* Δ6-12 phyA (in the *phyA* background) revealed a weak germination response to a pulse of long-wavelength FR (VLFR) and there were no significant differences among WT, full-length, and Δ6-12

phyA lines suggesting that phyA′ does not play a significant role in the regulation of seed germination.

Several signal transduction components downstream of phyA participating in germination were detected (Kim et al. 2008; Oh et al. 2004; Kneissl et al. 2009). Of interest in the context of our discussion are the *Arabidopsis* mutants deficient in FHL, FHY1, and related proteins — the phyA partners transporting it into the nucleus (Zeidler et al. 2004; Hiltbrunner et al. 2005). Mutations of *fhy1* and *fhy3* weaken the HIRs (Whitelam et al. 1993); *fhy3-1* retains VLFR but is severely impaired in HIR (Yanovsky et al 2000). Casal et al. (2014) suggest that the mechanism controlling the expression of genes *FHL/FHY1* operates under HIR but not under VLFR. We have undertaken experiments on *Arabidopsis fhl/fhy1* double mutant seeds with the aim to see if there is any connection of the mutation with the state of the phyA pools in the mutant seedlings. In these experiments, germination in the mutant reached 100% after 15 min of W illumination, whereas in the WT, not more than 10%; the WT seeds germinated with the rate of 100% only after 1 h exposition (L. Koppel, M. Zeidler and V. Sineshchekov, unpublished results). There were no differences in the total phyA content and the phyA′/phyA″ proportion in plant tissues suggesting that the phyA pools' modification is not connected with the phyA-FHL/FHY1 complex formation and that the observed germination effects are not connected with the changes in the phyA state.

Our experiments on *Arabidopsis* overexpressing fern *Adiantum capillus-veneris* phy1 (Sineshchekov et al. 2014a) may have a relation to the understanding of the specificity of the phyA state and action in seeds. The seeds of this transgenic line were characterized by an unexpectedly high germination rate in darkness and after the light pretreatment of 15 min and 3 h (Fig. 6), whereas phy1 was shown earlier to be inactive in seedling growth responses (Okamoto et al. 1997). The latter agrees with our observation that the overexpressed phy1 does not contribute to the stimulation of the phyA′ accumulation in seedlings upon seed preillumination (see above and Fig. 5). Given that phy1 is represented also by the two native pools like those of phyA (phy1′ and phy1″), we may speculate that phy1″ participates in this germination effect as does phyA″.

Phytochrome A photoregulation reactions in seedling growth and establishment

Phytochromes A and B are deeply involved in the regulation of plants' de-etiolation and seedling establishment, their action being overlapping and redundant, and, depending on the illumination conditions, synergistic or antagonistic (Reed et al. 1994; Casal 2000; Tepperman et al. 2006). phyB is more important under R and W light whereas phyA is a major photoreceptor for the FR-induced responses working in the VLFR and HIR modes. phyA mediates the regulation of growth reactions (Yanovsky et al. 1997; Casal 2000), anthocyanin accumulation (Duek and Fankhauser 2003), CAB biosynthesis induction, and block of greening (Reed et al. 1994; Barnes et al. 1996). It also suppresses gravitropism and stimulates phototropism (Sullivan et al. 2016).

In transgenic plants, the manifestation of the photoresponses depends on the presence of endogenous phytochromes (phyA and phyB) in the host plant, in which phyA, from a mono- or dicot plant, is introduced, and the promoter used. Transgenic plants expressing *Avena* phyA have shown exaggerated sensitivity to the VLFR conditions, when expressed in rice (Clough et al. 1995), tobacco (Casal et al. 1994; Sineshchekov et al. 1999b), and *Arabidopsis* (Mazzella et al. 1997). However, more often, the expression of monocot phyA in dicot plants revealed higher sensitivity to the FR-HIR conditions (Boylan and Quail 1989; Cherry et al. 1991; Halliday et al. 1999). The overexpression of an *Arabidopsis PHYA* in rice or soybean *PHYA* in *Arabidopsis* resulted in exaggerated FR-HIRs in growth inhibition by both Rc and FRc (Garg et al. 2006; Wu et al. 2013).

In our works, experimental evidence is being accumulated that the two modes of FR action, VLFR and HIR, are initiated by the different phyA types, phyA′ and phyA″, respectively. It was found that transgenic *Arabidopsis* plants (in the *phyA* and *phyAphyB* background) that expressed mutant rice phyA SA represented primarily or exclusively in the phyA″ form (Sineshchekov et al. 2019) were characterized by the HIR of growth responses (inhibition of hypocotyl elongation and anthocyanin accumulation under FRc) (Kneissl et al. 2008). At the same time, the wild-type rice phyA, which is represented by both phyA types, phyA′, and phyA″, complemented the wild-type *Arabidopsis* phenotype under FRp (VLFR) and FRc (HIR). Collectively, this allows the attribution of the HIRs to phyA″.

On the other hand, the Δ6-12 phyA, which is represented by phyA′ (Sineshchekov et al. 2014c), is likely to be responsible for the VLFR. Casal et al. (2002) have shown that oat Δ6-12 phyA expressed in transgenic tobacco and *Arabidopsis* was active in the VLFR of hypocotyl growth inhibition and cotyledon opening whereas the HIR responses were suppressed. Similarly, with the use of a homological system — *Arabidopsis* with the Δ6–12 deletion in *Arabidopsis PHYA* — it was shown that the 6–12 aa. region is dispensable for the VLFRs but is necessary for the HIR (Trupkin et al 2007).

Less defined are our results on the assignment of the phyA pools to the different response modes obtained with transgenic potato with modified phyA content (Sineshchekov et al. 1996) and transgenic wheat overexpressing oat phyA (Sineshchekov et al. 2001b). The uncertainty is because of the presence of the two phyAs in these lines. Based on a positive correlation between the manifestation of the HIRs

in growth responses and variations of [phyA′] (primarily in the potato and exclusively in the wheat), the HIRs were attributed to phyA′. However, the participation of phyA″ in the phenotype of these plants cannot be excluded because a relatively moderate increase of [phyA] (2–threefold) brings about a saturation of its action (Boylan and Quail 1989). On the other hand, phyA responses can be substantially modified in transgenic plants (Casal et al. 2002). We may speculate in this connection that phyA′ in overexpressors can acquire the properties and functions of phyA″. This assumption is supported by the observation (Clough and Vierstra 1997; Sineshchekov et al. 2001b) that overexpressed phyA is relatively light-stable which is characteristic of phyA″ (see discussion in (Sineshchekov 2019)).

Mutations in the phyA molecule, which do not affect the formation of the phyA pools, have an impact on the manifestation of the different photoresponse modes, most pronounced in the case of the HIRs. The amino acid substitutions R194V and C581T in pea phyA increased the de-etiolation phenotype under FRc and Rc without violation of the phyA′/phyA″ balance (Weller et al. 2004; Sineshchekov and Weller 2004). Mutant plants expressing phyA with the substitutions E777K and R384K become insensitive to or severely impaired in the HIR (Yanovsky et al. 2002; Mateos et al. 2006).

Of interest is the fact that transgenic *Arabidopsis* expressing oat phyA with a reduced kinase activity reveal limited photoresponses to FR — both HIR and VLFR (Shin et al. (2016), whereas those with an enhanced phyA kinase activity are hypersensitive under FR (short hypocotyls and expanded cotyledons) (Hoang et al 2021).

Mutations at S8A and S18A in oat phyA expressed in *Arabidopsis*, at the sites involved in the phyA autophosphorylation, bring about hypersensitivity to FRc and FRp, which is interpreted to result from the higher light stability of the mutated phyA (Han et al. 2010). There were no significant changes in the phyA′/phyA″ ratio in this *Arabidopsis* line suggesting that the effect is not connected with changes in their content but, rather, in their higher stability (Sineshchekov et al. 2021a). Boylan and Quail (1989) pointed to the higher stability of the heterologous (oat) phyA than the endogenous tomato phyA in transgenic tomato plants characterized by a higher de-etiolation phenotype.

The character of the phyA growth reactions may strongly depend on its functional interaction with the hormonal system as revealed by the experiments with rice mutants (*hebiba* and *cpm2*) (Riemann et al 2003, 2013; Sineshchekov et al 2004b, 2021a). For the WT rice, there is a differentiation of the photoregulation effects on different organs: the growth of coleoptiles and seminal roots is inhibited by the VLFR conditions, whereas for mesocotyls, by FR-HIR (Takano et al 2001; Shimizu et al. 2010). The JA mutants, however, show a sign reversal in these effects.

Whereas WT coleoptile growth is elevated in the dark and efficiently inhibited by R and FR, mutant coleoptiles are arrested in growth if they remain in the dark but expand rapidly upon illumination (Fig. 7). Our recent experiments on *hebiba* and *cpm2* (Sineshchekov et al. 2021a) have confirmed this sign reversal, in particular, phyA suppresses roots' growth under FRp in the mutants but not in the WT. They also have shown that the manifestation of a photoresponse depends on the age of the seedlings and on the illumination conditions (Fig. 7). For instance, the coleoptiles remain unresponsive to all the light regimes which are explained in agreement with Xie et al. (2007) and Shimizu et al. (2010) by the earlier age of the seedlings as compared to that in Sineshchekov et al. (2004b). Collectively, the data on JA mutant features suggest that JA reduces the phyA functional activity primarily in its phyA″ form mediating the HIRs.
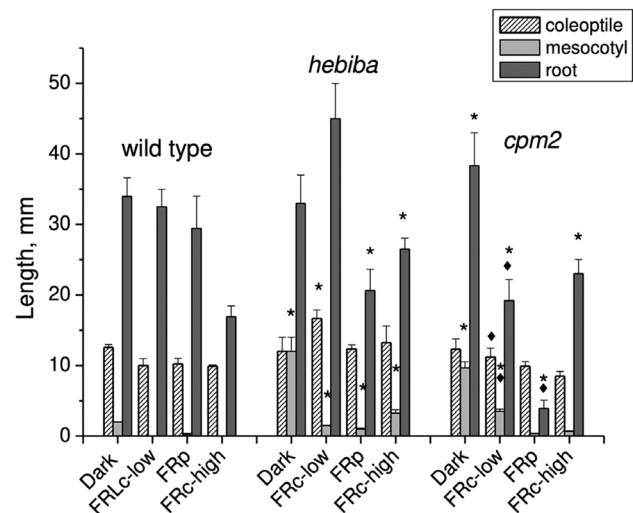
Fig. 7 The length of the coleoptiles, mesocotyls, and roots of dark-grown 5-day-old rice (*Oryza sativa* L. ssp. Japonica cv. Nihonmasari) seedlings and of its *hebiba* and *cpm2* mutants deficient in the hormone jasmonic acid (JA) and of the seedlings of the same lines grown under far-red light (see light conditions in the caption to Fig. 4). Statistically significant light effects are seen in the wild type in the case of mesocotyls; roots undergo significant growth inhibition only under FRc-high (Student's t-test; $P < 0.05$). Statistically significant light effects are seen: in *hebiba*—for coleoptiles under FRc-low, mesocotyls under all the light conditions, and the roots under FRp; in *cpm2*—for coleoptiles under FRp and FRc-high, and mesocotyls and roots under all the light conditions. The asterisks indicate the values of the mutants, which are significantly different from those of the wild type, and the diamonds point similarly to the values of *cpm2* significantly different from those of *hebiba*. (From Sineshchekov et al. 2021a)

The role of phytochrome A in the regulation of (proto)chlorophyll biosynthesis

The phyA heterogeneity may also have a close relationship to the complexity of the regulation of chlorophyll (Chl) biosynthesis and the formation of photosynthetic structures (see the review Sineshchekov and Belyaeva 2019). One of the outcomes of this regulation is the synchronization of the accumulation of potentially dangerous photoreactive pigment species with the formation of the protein matrix providing effective channeling of excess light energy into heat. The Chl biosynthesis path comprises two major steps: (1) the dark stage — from the synthesis of aminolevulinic acid (ALA) to the appearance of protochlorophyllide forms (Pchlide^{633} and Pchlide^{655}), and (2) the light stage — photoreduction of Pchlide^{655} into chlorophyllide, Chlide. Pchlide^{633} is a dark precursor of the active Pchlide^{655}; it is incapable of photoconversion into Chlide but is likely to be the major cause of the appearance of harmful reactive oxygen species. Pchlide^{655} is a component of the tripartite complex comprising also photoenzyme protochlorophyllide oxidoreductase (POR) and NADPH (Pchlide–POR–NADPH) capable of photoconversion into Chlide. The photoconversion of Pchlide^{655} into Chlide is a complex process comprising two successive photoreactions and several dark stages. phyA was shown to be involved in the regulation of both the dark and the light stages of the process — from ALA to Pchlide^{655} and from Pchlide^{655} to Chlide.

Phenomenological manifestations of the regulation of the (proto)chlorophyll biosynthesis by phytochrome A include positive and negative effects and are experimentally observed as (1) potentiation of greening — the reduction of the lag of chlorophyll synthesis by short (hours) periods of FR before the transfer of a seedling to W (Lifschitz et al. 1990; Yanovsky et al. 1997) and (2) blocking of greening by prolonged FR (days) illumination of a seedling before transfer to W (Apel 1981; Batschauer and Apel 1984; Runge et al. 1996; Barnes et al. 1996; Armstrong et al. 1995; Luccioni et al. 2002). The modes of the phyA photoregulation of these effects fall well into the two categories — the VLFRs and HIRs.

Yanovsky et al. (1997, 2000) observed both the VLFR and HIR of potentiation of the greening of etiolated seedlings of *Arabidopsis* Ler ecotype. Also, in the experiments on Chl biosynthesis blocking on a *fhy3-1* mutant, they showed that it retains the VLFRs but is severely impaired in the HIRs suggesting divergent signal pathways for the two modes of action in the Chl biosynthesis regulation.

Blocking of greening under FRp and FRc was followed with the use of *Arabidopsis* impaired in PKS1 or PKS2 (phytochrome kinase substrate) — *pks1*, *pks2*, and *pks1*

pks2 double mutants and transgenics overexpressing the PKS1 or PKS2 gene in (Lariguet et al. 2003). *pks1* and *pks2* showed enhanced responses to FRp but not to FRc. These results indicate that PKS1 and PKS2 specifically affected the VLFR pathway of phyA but not the HIR of phyA. This contrasts with the effect of the *fhy3-1* mutation, which affects the HIR but not the VLFR (Yanovsky et al. 2000). This is yet another argument for the notion of the distinct pathways of the two modes of phyA-mediated regulation.

In our experiments, it was confirmed that the phyA regulation of Chl synthesis is primarily the HIR and that phyB may interfere with the action of phyA. In coleoptiles of WT rice and its *phyB* mutant grown under FRc and FRp, the effect of the light treatment was evaluated by direct measurements of Pchlide^{633} and Pchlide^{655} fluorescence proportional to their content in tissues (Sineshchekov et al. 2006). Under FRp (the VLFR conditions), there were no significant changes in the levels of both forms of protochlorophyllide in the WT, whereas a considerable decrease was observed for Pchlide^{633} (40%) and Pchlide^{655} (ca 100%) in the *phyB* mutant. In seedlings grown under FRc (the HIR conditions), the inhibitory effect of phyA on [Pchlide^{633}] was much higher — [Pchlide^{633}] decreased by threefold in WT and by sevenfold in the *phyB* mutant, and [Pchlide^{655}] was almost completely absent in both the lines.
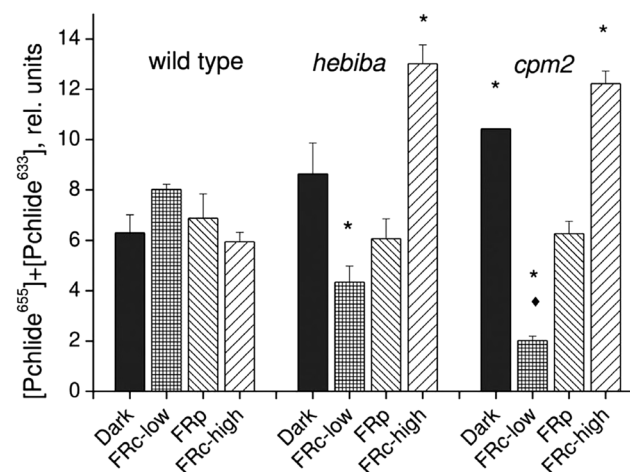
Kneissl et al. (2008) confirmed that phyA is responsible for suppressing Pchlide accumulation under FR using *Arabidopsis* deficient in phyB and both phyA and phyB and that phyB negatively affects the action of phyA. Of particular importance in the context of the current review is their observation that the rice mutant phyA SA ectopically expressed in *phyB* or *phyAphyB Arabidopsis* was considerably less efficient than the WT rice phyA in the Pchlide biosynthesis suppression under FRp (VLFR), whereas the effect of FRc (HIR) was similar in both the lines. Given that phyA SA is represented primarily or exclusively by the phyA″ species (i.e., lacking phyA′) (Sineshchekov et al. 2018), the above observation indicates that phyA″ is responsible for this HIR effect and phyA′, for the VLFR, and that phyB suppresses the phyA′ action.

In line with this assignment of the two different modes of the Chl biosynthesis regulation by phyA to the two phyA pools are the data on Δ6-12 phyA of oat expressed in *Arabidopsis* (Casal et al. 2002) The truncated phyA was hyperactive for the FRp blocking greening upon transfer to W in *Arabidopsis* (the VLFR), whereas the effect of FRc (the HIR) was reduced compared with the full-length (FL) phyA. Trupkin et al. (2007) have carried out similar experiments in a more physiological context — on transgenic *Arabidopsis* expressing Δ6-12 *Arabidopsis* PHYA. They showed normal responses to FRp and impaired responses to FRc. Besides, reduced light stability of deleted phyA was observed and

the authors explain the reduced phyA physiological activity by the enhanced destruction of the mutated phyA. The data by Casal et al. (2002) can be thus straightforwardly explained as a manifestation of the functions of the phyA′ type — the dominating or the only phyA species present in the transgenic *Arabidopsis* expressing Δ6-12 (oat) phyA (Sineshchekov et al. 2014c). This may hold also in the case of Δ6-12 phyA of *Arabidopsis* (Trupkin et al. 2007) because of the location of the deletion and the fact that the truncated phyA has higher light lability what is the property of phyA′.

The phyA regulation of Chl biosynthesis and its dependence on the cellular context are becoming even more complicated by the fact that its sign and extent depend on plant species and organ/tissue (Sineshchekov et al. 2004a). Pchlide^{655} content in the upper stems of FRc-grown seedlings of pea and tobacco increased approx. tenfold as compared with the dark-grown. In the upper stems of *Arabidopsis* and tomato, the positive effect of FRc was low, 1.2- to 1.5-fold, and the negative effect of FR was seen in cotyledons. The regulation of Pchlide^{633} in contrast to Pchlide^{655} was positive independent of the plant species and tissue.
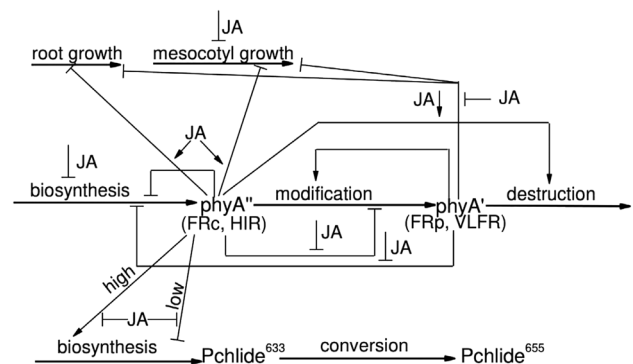
More to that, the phyA regulation of Chl accumulation depends on the hormonal status of the plant. In experiments on coleoptiles of rice *hebiba* deficient in JA, the content of Pchlide^{633} and Pchlide^{655} in the dark was higher in the mutant pointing to the inhibitory effect of the hormone on their biosynthesis in the WT. In seedlings grown under FR, the sign of the Pchlide^{655} regulation was found to depend on the mode of the light action (VLFR or HIR). In the wild type, FRp (VLFR) was stimulating whereas FRc (HIR) inhibiting (Fig. 8). In the mutant, both FRp and FRc stimulated the Pchlide^{655} biosynthesis, i.e., the sign of the FRc effect has changed from the negative in the WT to the positive in *hebiba*. Under FRc, [Pchlide^{633}] remained the same as in the dark in the WT and grew in *hebiba*.

In our recent experiments on the same mutant *hebiba* and a similar mutant line *cpm2,* we observed a different picture (Sineshchekov et al. 2021a). The proportion of the two Pchlide species was rather conserved, it varied within a very narrow limit. So, the FR effects were evaluated by changes in the total Pchlide content, [Pchlide^{633}] + [Pchlide^{655}]. In the WT, the FRc and FRp effects were insignificant, whereas in the mutants FRp was inhibiting and FRc stimulating (Fig. 8). This is at variance with the data on the WT rice (of a different var.) in (Sineshchekov et al. 2006), when FRc brought about a complete block of [Pchlide^{655}] and a considerable decline in [Pchlide^{633}], and on *hebiba* in (Sineshchekov et al. 2004b), when the FR effects were different for the two Pchlide species. The reversion of the sign of the FRc effect was also observed in the mutant (see above). This variability of the FR effects on Chl synthesis even in the same plant (rice) suggests their dependence on the physiological status of the plant, possibly, on its age. Differences in the illumination regimes and light spectrum may also affect the observed results (see the discussion in Sineshchekov et al. 2021a). Thus, phyA can differentially affect the biosynthesis

Fig. 8 The content of the two protochlorophyllides ([Pchlide^{655}] + [Pchlide.^{633}]) in the coleoptiles of the wild-type seedlings of rice (*Oryza sativa* L. ssp. Japonica cv. Nihonmasari) and its *hebiba* and *cpm2* mutants. The pigments were determined by fluorescence emission spectra. The effects of the illumination of the different regimes on the protochlorophyllides' content are not statistically significant in the wild type but they are in the mutants (indicated by an asterisk). For the illumination conditions, see the legend in Fig. 4. (From Sineshchekov et al. 2021a)

Fig. 9 Hypothetical model of the relationship between phytochrome A pools (phyA′ and phyA″) and phytohormone jasmonic acid (JA) signaling in etiolated 5-day-old seedlings of rice grown under far-red illumination (FR) of different regimes — FR constant (FRc, the HIRs) and FR pulsed (FRp, the VLFRs). Thin arrows indicate signaling and thicker arrows, metabolic processes. "High" and "low" stand for the light signaling under FRc of different (by approx. 50-fold) fluences. Lines with arrowheads correspond to positive regulation, lines with blunt ends, to negative regulation. As one can see from the scheme, almost all the effects of JA on the light signals from phyA′ (FRp, VLFR) and phyA″ (FRc, the HIRs) are inhibiting, except for the three effects, when JA promotes phyA signaling – the phyA″ inhibition of phyA biosynthesis and root growth, and the stimulation of phyA′ destruction. Coleoptiles' growth is not affected because of seedlings' young age. (From Sineshchekov et al. 2021a)
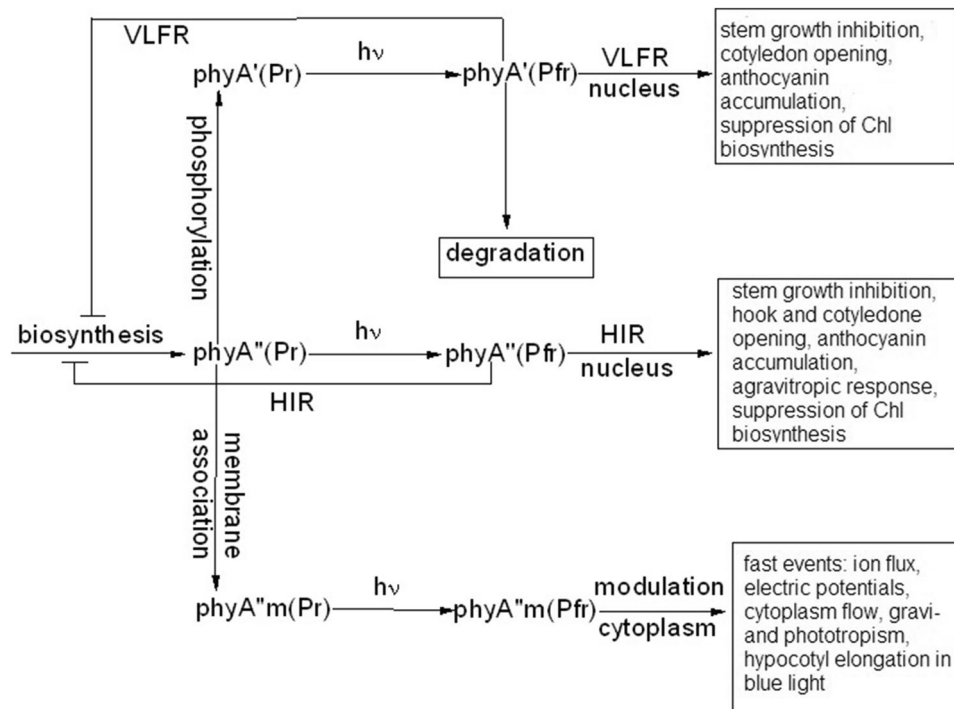
Fig. 10 Working scheme of the state and functions of the native phytochrome A pools in etiolated seedlings. In seeds, phyA is likely to be present in the phyA″ form and participates in the regulation of seed germination. De novo synthesized phyA in germinating seeds and growing seedlings is initially in the phyA″ form, which possesses amphiphilic properties and is present in the cell in water-soluble and membrane- (protein-) associated (phyA″m) fractions. In darkness, phyA″ is converted into the water-soluble phyA′ form, possibly, via serine phosphorylation at the N-terminus of the molecule. Upon illumination, the water-soluble phyA′ and phyA″ are transported into the nucleus forming there two different types of nuclear speckles and inducing different modes of photoresponses, the VLFRs and HIRs, respectively. phyA″m in the Pfr form remains in the cytoplasm and initiates fast biochemical processes. Pointed arrows indicate the stimulation effects; blunt-ended arrows indicate the inhibitory effects

of Pchlide under the VLFR and HIR conditions, and JA counteracts this action in WT (Fig. 9). The suppression of the phyA action by JA may include phyA destruction (see above), and the modulation by JA of the level of the phyA transporters into the nucleus — FHY1 and FHL (Liu and Wang 2020). In general, our data on phyA regulation of Chl biosynthesis in the JA mutants are in line with the notion that the signals from phyA and JA are mutually antagonistic (see reviews Hsieh and Okamoto 2014; Sineshchekov and Belyaeva 2019).

To the functions of phyA in the cytoplasm

The difference in the hydrophilicity/hydrophobicity of the two phyA pools (see above) may relate to their functional activity. Given that the cytosol is also the site of phyA action (see discussion in Rösler et al. 2007; Jaedicke et al. 2012; Hughes 2013), we may hypothesize that the membrane-(protein-) associated fraction of phyA″ (phyA″m) is the most likely candidate for the initiation of the phyA-specific cytoplasmic responses. Rösler et al. (2007) have shown that the phyA-induced cytosolic photoresponses — R-enhanced phototropism, abrogation of gravitropism, and inhibition of hypocotyl elongation in blue light — are essentially the same in the WT *Arabidopsis* and in the *fhl/fhy1* mutant defective in the light-induced nuclear import of phyA, that is they are identified as phyA-specific cytoplasmic responses. In our opinion, the soluble phyA′ and phyA″ fractions participate in the nuclear regulatory effects (see above), whereas phyA″m, in the cytosolic. This notion is supported by the fact that a small fraction of the phytochrome population is associated with phototropin and with PKS1, and that this association is required for phototropism (Lariguet et al 2006; Jaedicke et al 2012). It is tempting to hypothesize that the phyA″m pool could be responsible for the fast photoregulation effects in the cytoplasm, such as modulation of ion transport, electric potentials, and cytoplasm fluidity. This needs, however, direct experimental verification.

Conclusions

Phytochrome A grants plant's survival under the conditions of deep vegetative shade when the ambient light is shifted towards domination of the far-red, photosynthetically impotent component. Its regulatory action critical at the very early stages of plant development includes two modes of the FR-induced photoresponses — the VLFRs and HIRs. This complex character of the phyA action is due, at least, partially, to the structural heterogeneity of the photoreceptor — the existence of its two native types, phyA′ and phyA″. phyA is synthesized in the cytoplasm in the unphosphorylated phyA″ form, which converts into phyA′, possibly, via serine phosphorylation at the NTE. phyA′ is water-soluble whereas phyA″ reveals ambiquitous properties — it is found in the liquid phase and associated with a membrane (phyA″m). The three different native phyA species correlate with the three distinct phyA complex formations observed after its light-induced nucleocytoplasmic partitioning — two types of speckles in the nucleus and a cytoplasmic fraction. In seeds, phyA is likely to be present primarily in the phyA″ form, where it participates in the regulation of seed germination. In seedlings, the two phyA species mediate different modes of photoresponses acting in the nucleus — phyA′ initiates the VLFRs, and phyA″, the HIRs, and, probably, the LFRs (Fig. 10). phyA″m may be responsible for the cytosolic activity of phyA including the regulation of photo- and gravitropism In this review, this attribution is demonstrated by the key photoregulation processes — light induction of seed germination, growth responses during de-etiolation, and Chl biosynthesis.

The sign and extent of the phyA photoresponses may depend on the plant's species and organ/tissue, its developmental state, and hormonal status. This is evident, in particular, in the case of the phyA regulation of Chl synthesis when, under certain conditions, instead of its suppression there was its stimulation. The reversion of the sign of the phyA regulation of Pchlide^{655} accumulation was also observed in the rice mutant *hebiba* deficient in JA. It differentially affects the two phyA response types, the HIRs (mediated by phyA″) and the VLFRs (mediated by phyA′), reducing primarily the phyA″ effects (HIR). This may relate to JA participation in phyA destruction and suppression of the FR-induced phyA transport into the nucleus.

Several key questions in the concept of the structural and functional heterogeneity of phyA remain yet to be solved. This is, first, exact chemical differences between phyA′ and phyA″ granting their physicochemical and functional distinctions. The most straightforward approach here would be to get the two pools separately in a heterologous system and investigate them in vitro with the use of analytical chemistry and crystallography. Second, the exact functions and the mechanisms of action of the three native phyA pools are to be further investigated with the use of transgenic plants or mutants deficient in phyA′ or phyA″. And finally, this structural and functional heterogeneity of phyA is to be considered while exploring its interactions with the hormonal system.

Declarations

Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate Human subjects were not involved in the research.

Consent to publication Individuals were not involved in this study.

Competing interests The authors declare no competing interests.

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