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REVIEW

Regulation of Chlorophyll Biogenesis by Phytochrome A

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Abstract—The photosynthetic apparatus accomplishes two major functions in plants – solar energy conversion and protection of the plant from photodestruction. Its highly orchestrated formation includes coordinated biosynthesis of chlorophyll (Chl) and of its binding to matrix proteins. Light plays here the central role driving both metabolic and regulatory processes. The regulation is achieved via operation of sophisticated photoreceptor machinery with the phytochrome system as its main component. This review concentrates on Chl *a* biosynthesis and the role of phytochrome A (phyA) in this process. The mechanism of action of phyA and the specificity of its state in the plant has been described, in particular, the existence of two native types with different modes of action. This review touches upon the dependence of the effects of phyA on tissues and organs of the plant and its species, genetic modifications, and hormonal status.

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Dedicated to Professor F. F. Litvin on the occasion of his 90th birthday

As sessile organisms, autotrophic plants possess a vital capability to adapt their growth and development to the environmental light conditions. The strategy of such adaptation is to maximize solar energy conversion and minimize photochemical damage. These two goals are achieved already on the molecular level with the involvement of photophysical and photochemical processes, first of all of excitation energy migration. This increases the absorption cross-section and light-harvesting capacity and channels excess excitation energy to specialized forms of chlorophyll (Chl) and carotenoids dissipating it into heat.

At the stage of formation of the photosynthetic apparatus, when the mechanism of energy transfer and dissipation is not fully operating, photodamage may take place connected with the appearance of highly reactive free (proto)chlorophyll(ide) molecules. Plants minimize this negative effect by down-regulation of their accumulation and by the promotion of their incorporation into photosynthetic structures with the use of sophisticated light perception machinery. A pivotal role in this regulation belongs to the phytochrome system with phytochromes A and B (phyA and phyB) as the major actors. They coordi-

Abbreviations: ALA, δ-aminolevulinic acid; ALAD, ALA dehydratase; BRs, brassinosteroids; Chl, chlorophyll; Chlide, chlorophyllide; CS, chlorophyll synthase; ERS, endogenous regulation signals; ET, ethylene; FHY1, FHY3 and FHL, partner proteins of phytochrome A (far-red elongated hypocotyl 1 и 3, FHY1-like); FR, far red light; FRc, continuous FR; FRp, pulsed FR; Glu TR, Glu tRNA reductase; HIR, high irradiance responses; JA, jasmonic acid; LFR, low fluorescence responses; MC, Proto IX MME cyclase; MgCh, Mg-chelatase; MgPPMT, Mg-Proto IX methyltransferase; Mg-Proto IX, Mg-protoporphyrin IX; Mg-Proto IX MME, Mg-protoporphyrin IX monomethyl ester; NTE, *N*-terminal extension; PBG, porphobilinogen; Pchl, protochlorophyll; Pchlide, protochlorophyllide; phyA(B,C), phytochrome A(B,C); phyA′ and phyA″, native pools of phyA; PIFs, phytochrome-interacting factors; PLB, prolamellar bodies; PORA(B,C), protochlorophyllide oxidoreductase A(B,C); Proto IX, protoporphyrin IX; Pr and Pfr, phytochrome forms absorbing red and far-red light respectively; PSI(II), photosystem I(II); R light, red light; RS, retrograde signal; VLFR, very low fluorescence responses.

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nate the rates of the synthesis of both Chl and the binding proteins in the formation of stoichiometrically organized light-harvesting complexes [1]. phyA plays a leading role in early seedling photomorphogenesis and gene expression, whereas phyB dominates in mature plants. phyA exclusively ensures regulation of plant photomorphogenesis under conditions of FR (far red light) domination, in particular, in the shade of dense canopies. This process is believed to have contributed to the evolutionary advantages of higher plants [2, 3].

Under FR-enriched light, when the light-induced protochlorophyllide (Pchlide)-to-chlorophyllide (Chlide) conversion is slowed because of their low absorbance in this spectral region, phyA downregulates accumulation of active Pchlide, which can undergo photoconversion into Chlide [4-6]. At the same time, data are being accumulated on the positive regulatory effect of phyA under FR on the active Pchlide content [7-9]. Of interest is also the fact that the sign of the FR effect on active Pchlide formation – promotive or inhibitory – depends on the hormonal status of the plant [10]. Besides, existence of the two phyA populations with different photoresponse modes – the very low fluorescence responses (VLFR) and high irradiance responses (HIR) [11, 12] – makes these regulatory processes even more complex. In general, the problem of the regulatory interaction between phyA and hormonal and other plant signaling systems is acquiring great importance. Collectively, all this prompted us to try to analyze phytochrome regulation of Chl biosynthesis with emphasis on the unique role of phyA in this process. The theme of the review and its scope were inevitably predetermined by the scientific interests of the present authors which have been formed under the influence of Prof. F. F. Litvin and his pioneering works on the photobiophysics of photosynthesis and spectroscopy of plant pigments (see below). This circumstance and the limited volume of the review did not allow us to adequately present all the relevant original papers, and the reader is referred to the recent comprehensive reviews related to a much broader issue of photomorphogenesis and photosynthesis regulation in plants (see reviews [13-20]).

BIOGENESIS OF THE LIGHT-HARVESTING APPARATUS OF PHOTOSYNTHESIS. SPECTRAL FORMS OF Pchlide AND Chl(ide)

The process of Chl biosynthesis and biogenesis of its native forms is separated into a dark stage, formation of Pchlide from its precursor δ-aminolevulinic acid (ALA) (see below), and a light stage, photoconversion of Pchlide into Chlide and further into Chl and its various forms. Pchlide is a Mg^{2+}-containing cyclic tetrapyrrole that differs from Chl by the lack of two hydrogen atoms at position 17-18 of the fourth pyrrole ring (ring D). Thus, the terminal light step in Chl biosynthesis is a photochemical hydrogenation of the semi-isolated double bond C17=C18 in the Pchlide molecule. The light stage is characteristic for higher plants and some cryptogams and gymnosperms. In cyanobacteria, green algae, cryptogams, and gymnosperms, the Pchlide-to-Chlide conversion proceeds in darkness.

During darkness, a substantial number of different Pchl(ide) forms appear as revealed by spectroscopic investigations of etiolated leaves *in vivo*. Approximately 10 spectroscopically different Pchl(ide) forms have been identified: $P^{633}/_{628}$, $P^{643}/_{637}$, $P^{655}/_{650}$, $P^{669}/_{657}$, $P^{682}/_{670}$, $P^{686\text{-}690}/_{676}$, $P^{697}/_{686}$, $P^{728}/_{696}$ (fluorescence/absorption maxima) [21-23]. However, the bulk pigment is represented by the first three forms: $Pchlide^{633}/_{628}$, $Pchlide^{655}/_{650}$, and $Pchlide^{643}/_{637}$ (Fig. 1a). $Pchlide^{643}/_{637}$ is virtually indiscernible in the fluorescence spectrum due to highly efficient energy migration from this form to $Pchlide^{655}/_{650}$.

$Pchlide^{655}/_{650}$ and $Pchlide^{643}/_{637}$ are converted to Chlide upon brief illumination. The short-wavelength form $Pchlide^{633}/_{628}$ converts into Chlide under specific light conditions [24-27] and is likely to be a precursor of the active $Pchlide^{655}/_{650}$ [28, 29]. $Pchlide^{633}/_{628}$ is a form that is not protected from photooxidation because of its monomeric nature or the lack of adjacent carotenoids [25]. The dominating active $Pchlide^{655}/_{650}$ is the main precursor of the Chl forms of the light-harvesting complexes of the two photosystems. It comprises two subpools consisting in $Pchlide^{653}/_{648}$ and $Pchlide^{657}/_{650}$: $Pchlide^{653}/_{648}$ being bound to protothylakoids or thylakoids, while $Pchlide^{657}/_{650}$ is bound to prolamellar bodies (PLBs) [29]. $Pchlide^{655}/_{650}$ exists either as a dimer or a tetramer, and the longer-wavelength Pchlide forms are supposedly large aggregates of the pigment [30]. These forms may serve as a sink of excess light energy received from the shorter-wavelength forms of the precursor, thus protecting them from photodestruction.

Of interest is the fact that the minor longer-wavelength $Pchlide^{686}/_{676}$ and $Pchlide^{682}/_{672}$ forms are involved in the biosynthesis of the reaction centers of photosystem (PS) II and PSI [31, 32]. Thus, the pigment–protein complexes of the Chl precursor display a high degree of plasticity, which is probably geared to optimize the adaptation to changing environments, particularly the prevailing light conditions.

The generation of the native Chl forms includes a number of light and dark reactions (sequential and branched, and also parallel and intersecting via identical intermediates), which lead to the formation of all the pigment–protein complexes of the reaction centers and light-harvesting complexes of the two photosynthetic systems [33]. Conversion of $Pchlide^{655}/_{650}$ into Chlide forms (Fig. 1b) includes two consecutive photoreactions and several subsequent dark steps. The first photoreaction leads to the formation of $Chlide^{684}/_{676}$, which is a mixed

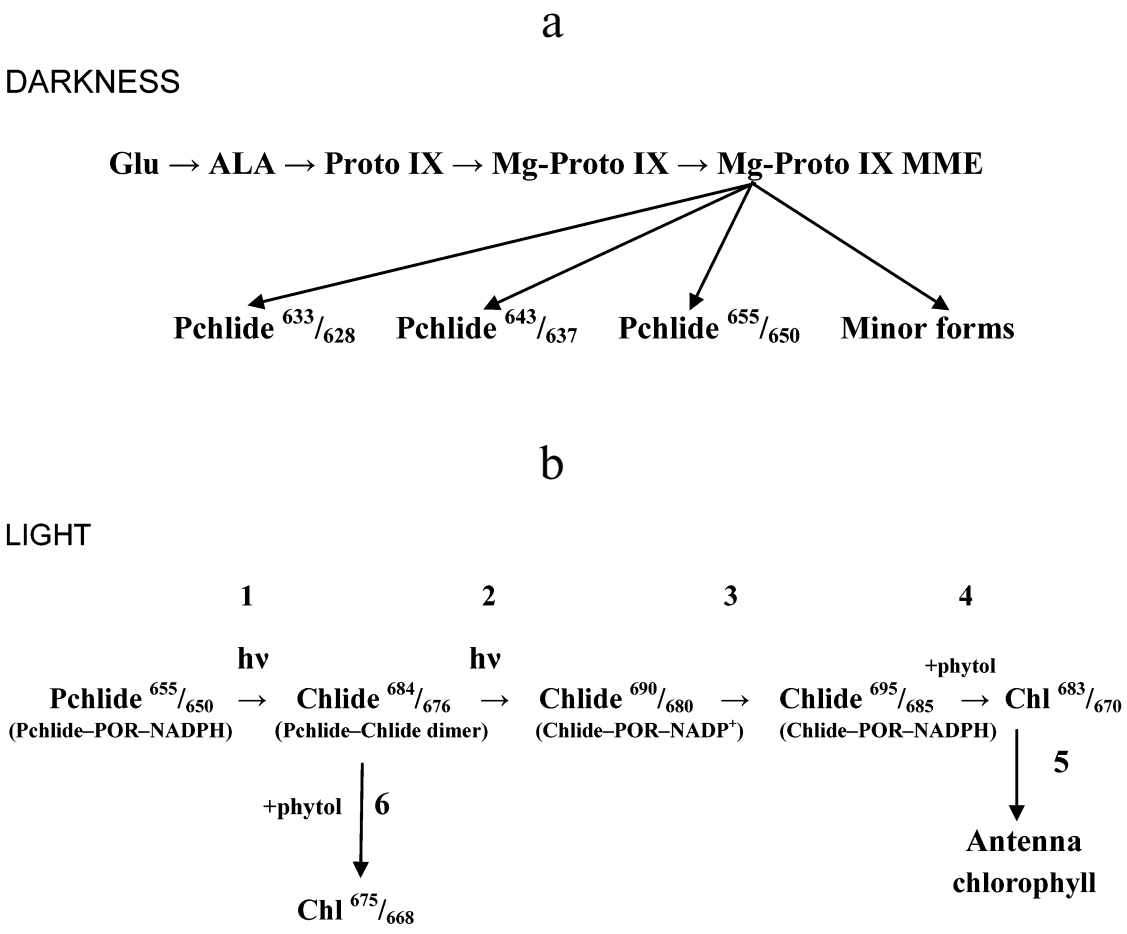

Fig. 1. Simplified scheme for the dark biogenesis of protochlorophyllide (a) and conversions of the pigment forms at the light stage of chlorophyll biosynthesis (b) in higher plants. The slash-separated numbers here and in the text below indicate the positions of spectral maxima (fluorescence/absorption) at low temperature (77 K) for various pigment forms (modified from [33]).

dimer Pchlide–Chlide. The second photoreaction completes photoreduction of this aggregate by formation of Chlide dimer or a tetramer – Chlide$^{690}/_{680}$. The temperature-dependent spectral shift Chlide$^{690}/_{680}$ → Chlide$^{695}/_{685}$ (reaction (3) in the scheme) is light-independent and is connected with changes of the pigment–protein complex comprising reduction of $NADP^+$ to NADPH. Reaction (4) is the so-called short-wavelength "Shibata shift" with the formation of Chl$^{683}/_{670}$, which is followed by protochlorophyllide oxidoreductase (POR) (see below) and Chlide disaggregation and attachment of phytol to yield Chl with the participation of chlorophyll synthase. The chlorophyll molecule leaves POR and moves to other proteins in the forming thylakoid structures. Further on, longer-wavelength Chl species are formed with the use of proteins synthesized during the dark stage of development. At the interface point of the two photoreactions (at the formation of Chlide$^{684}/_{676}$), there appears a side way leading to a shorter-wavelength Chl form Chl$^{675}/_{670}$. The esterification of chlorophyll occurs thus not only in association with the "Shibata shift," but it proceeds at a substantially (by an order of magnitude) higher rate in the dark reaction of Chl$^{675}/_{670}$ formation from the product of the first photoreaction. This reaction proceeds with a considerable rate only at temperatures above 273 K, and this pathway of Pchlide conversion dominates over the main one at low light intensity. All the above reactions take place within the first 30 min of illumination.

During the first 2-4 h after the onset of illumination, Chl accumulation is very low. However, activity of the photosynthetic systems is observed already in the first minutes of illumination of etiolated leaves, i.e., formation of reaction centers (RCs) and their immediate surroundings proceeds in etioplasts earlier than the accumulation of bulk Chl. During this period crucial rearrangements of the plastidic membranes take place, including grana formation [34, 35], and several Chl forms appear that differ from those in the light harvesting complex of mature plants [36]. Practically all the Chl forms that are characteristic for a mature leaf appear at the 4-6th hour after the

onset of illumination. By the 16th hour, a constant relative concentration of the pigment in the Chl forms is achieved characteristic for an adult green plant (of bean).

In the mature pigment apparatus, there exists an energy stairway formed by the excitation energy levels of the Chl forms [37, 38]. It provides 80-100% of absorbed quanta flow to the $Chl^{738}/_{698}$ form in PSI, whereas in PSII, the $Chl^{695}/_{690}$ and $Chl^{703}/_{698}$ forms are terminal energy acceptors in the case of blocked RCs (in bean). There exists also efficient backward energy transfer between the $Chl^{680}/_{676}$ and $Chl^{686}/_{682}$ forms and the energy migration pathways passing by one or two steps of the energy stairway. The energy transfer from carotenoids to Chl *a* takes place in a complex of the two pigments. The native Chl forms and the complex interactions between them thus provide directed energy flow to the RC in plants and their efficient protection from photodestruction.

Pchlide BIOSYNTHESIS AND ITS DARK REGULATION

Formation of Pchlide is accomplished in darkness as a multi-step process that is common for all plant tetrapyrroles (Fig. 2a). A universal precursor of all the cyclic (Chl, heme, corrinoids) and linear (bilins) tetrapyrroles is δ-aminolevulinic acid (ALA). In higher plants, algae, and a number of photosynthetic bacteria, ALA is formed from glutamic acid. Metabolism of plant tetrapyrroles is localized in plastids, and all the enzymes of the biosynthesis chain are coded by nuclear genes. The first key step in tetrapyrrole biosynthesis, ALA synthesis, includes three consecutive reactions, which are catalyzed by the enzymes glutamyl-$tRNA^{Glu}$ synthetase, glutamyl-$tRNA^{Glu}$ reductase, and glutamate 1-semialdehyde aminotransferase.

The second key step is the formation of protoporphyrin IX (Proto IX), which is a branch-point intermediate to hemes and Chls. Incorporation of magnesium into the porphyrin cycle with the formation of Mg-Proto IX is the initiation of the chain of the Chl branch of tetrapyrrole synthesis. The ATP-dependent reaction of chelation is catalyzed by magnesium chelatase (MgCh). Mg-Proto IX is converted to Mg-Proto IX monomethyl ester (Mg-Proto IX MME) by a Mg-protoporphyrin IX methyltransferase (MgPPMT).

The third key point in the process is the formation of the Pchlide from Mg-Proto IX MME. The synthesis of the Pchlide molecule is synchronized with the synthesis of the pigment-binding proteins. In etiolated plants, a prolamellar structure of etioplasts (PLB and prototylakoids (PT)) is formed, which comprises proteins containing Pchlide and functioning as photoenzymes in the reaction of Pchlide photoreduction into Chlide – protochlorophyllide oxidoreductases (PORs) [39]. It is important to mention that there are three POR species: PORA, PORB, and PORC (see below). Besides, PORs are proton donors in the Pchlide photoreduction [40]. The third component, which is necessary for the photoreduction of Pchlide into Chlide, is a hydride ion donor – NADPH [39]. The formation of the ternary photoactive pigment–protein complex comprising Pchlide, NADPH, and photoenzyme POR terminates the dark stage of Chl biosynthesis. At the dark stage of Chl biosynthesis, three key moments are to be balanced and regulated – accumulation of Pchlide, formation of a photoenzyme POR, and availability of a hydrogen donor NADPH.

Regulation of the stoichiometrically balanced ternary complex Pchlide–POR–NADPH takes place at the above key steps in the biosynthesis: on the formation of (i) ALA, (ii) protoporphyrin IX, and (iii) accumulation of Pchlide–POR–NADPH complex itself and heme. The regulation of ALA biosynthesis is accomplished to a great extent at the metabolic level via retroinhibition by the end products, primarily by the active Pchlide form, Chlide, and heme [41]. The mechanism of the metabolic signal is not well understood; however, it was shown that regulatory protein FLU participates in this process modulating the activity of the enzyme glutamyl-tRNA reductase 1 via interaction with its gene *HEMA1* [41] (Fig. 2a). McCormac and Terry [42] showed the important role of regulatory proteins GUN1 and GUN5 in independent pathways of feedback regulation of *Lhcb* and *HEMA1*. GUN1 is an important suppressor of *HEMA1* expression in the dark and under saturating white light. Besides, the retrograde signal suppresses transcription of GLK1, one of the main transcriptional regulators of photomorphogenesis [17, 43]. This metabolic signal participates in the signal transduction chain from plastids to the nucleus in order to regulate nuclear gene expression in accord with the functional state of the plastids. These reverse signaling processes from plastids to the nucleus are important not only in etiolated plants, but also in green plants for regulation of expression of the nuclear genes, where retrograde signal is the antagonist of phytochrome in the regulation of GLK1 [43]. There are several plastidic signal transduction chains that interact with each other and form a signaling net providing interconnection between the plastids and the nucleus [44].

The transition from scoto- to photomorphogenesis (de-etiolation) is the most vulnerable moment for a plant because accumulation of excess Pchlide not connected with POR, or other intermediates of biosynthesis, may lead to photodestruction of cells due to formation of reactive oxygen species or free radicals [45, 46]. Because of that, the main genes of tetrapyrrole biosynthesis are negatively regulated [14, 17, 18, 20, 47]. A crucial role in this regulation is played by phytochrome-interacting factors (PIFs), transcription factors endowed with a basic helix-loop-helix motif involved in broad cellular processes as a signaling hub integrating light, hormone, and other mul-

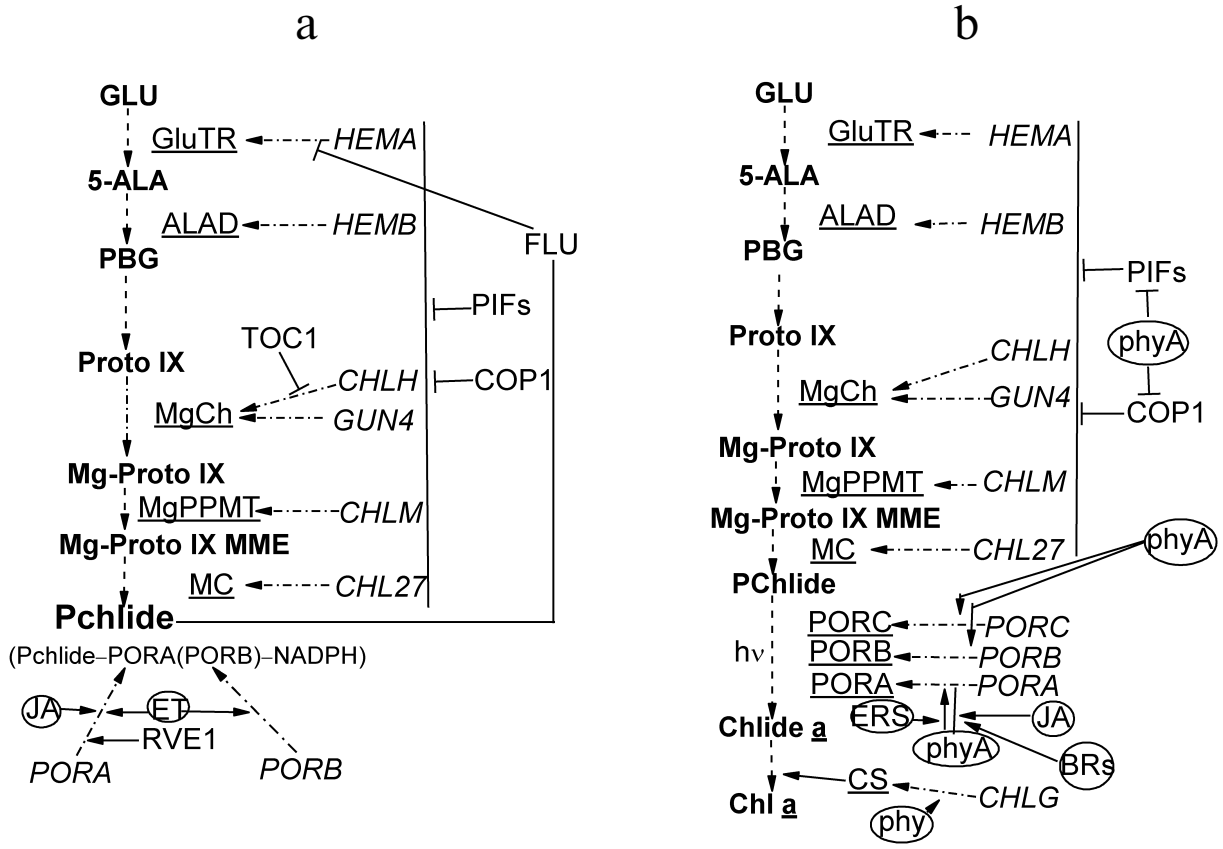

Fig. 2. Scheme of the Chl biosynthesis pathway and its regulation in higher plants in darkness (a) and in the light with the participation of phytochrome (b). Reaction substrates are given in bold, enzymes of key reactions are underlined, their respective genes are shown in italic, hormones and phytochromes are presented in ovals, protein factors participating in the regulation are given as plain capital letters. Dashed lines correspond to metabolic steps, dotted-dashed lines to gene expression, and solid lines to regulation (positive – with arrow-heads, negative – with blunt ends). Positive or negative regulation of *PORA* by phyA depends on hormones (JA and BRs) and on plant- and organ/tissue-specific endogenous regulation signals (ERS) (see *Abbreviations*).

tiple developmental signals [16, 48]. PIFs (PIF1, PIF3, PIF4, and PIF5) accumulate in the nucleus in the dark and repress photomorphogenic responses, including Chl biosynthesis.

Another key regulator of gene expression of tetrapyrrole biosynthesis (TPB) is the protein COP1, which negatively regulates protein HY5 – the factor stimulating transcription of practically all the genes coding for tetrapyrrole biosynthesis enzymes [49]. Besides, transcriptional factor TOC1 represses gene *CHLH*, coding for one of the three subunits of Mg chelatase by directly binding to the promoters of genes [50]. Gibberellins accumulated in the dark contribute to the activation of PIFs by degrading DELLA proteins and releasing PIFs from inhibition by DELLAs [51]. Thus, activation of negative regulators and inactivation of positive regulators could keep the expression of key *TPB* genes low in the dark. On the other hand, accumulation of sufficient quantities of POR, which binds free Pchlide molecules, provides defense of the plant against photodestruction. This is the reason for positive regulation of the *PORA* and *PORB* genes in etiolated seedlings, which proceeds with participation of the phytohormone ethylene and transcription factors COP1, EIN3, ABI4, and RVE1 [18, 52].

LIGHT REGULATION OF Chl BIOSYNTHESIS. PHYTOCHROMES

Mechanism of action of phytochrome A (phyA). Two native phyA types. Upon transition to the light, the plant experiences principal rearrangement of its total biology including the regulatory mechanism with its numerous light-responsive genes coding for the key enzymes for biosynthesis of Chl and pigment-binding protein (Fig. 2b). Among the system of photoreceptors of different nature comprising the highly sophisticated apparatus of light signal perception in plants, phytochromes occupy the central position because of their functional importance and of their more profound investigation.

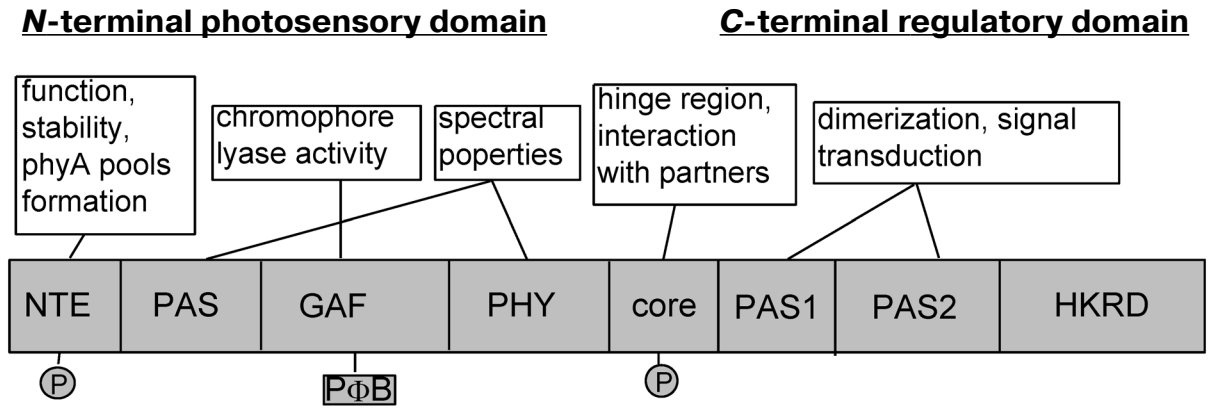

Fig. 3. Scheme of the phytochrome A molecule. It consists of two monomers comprising three domains: (i) the photoreceptor *N*-domain (70 kDa) containing four conserved subdomains – chromophore (phytochromobilin, PΦB) bearing GAF, PAS, and PHY implicated in defining spectroscopic and photochemical properties, and also the 10 kDa *N*-terminal extension (NTE) responsible for a number of key properties of phyA; (ii) the flexible hinge region connecting the *N*- and *C*-terminal domains, and (iii) the regulatory *C*-domain (55 kDa) with PAS1 and PAS2 subdomains participating in dimerization and signal transduction, and a histidine kinase domain (HKRD) whose functions are not defined.

Phytochromes represent a small gene family with phyA and phyB being the most important members. phyA is relatively abundant in etiolated tissues, which has allowed profound investigations *in vitro* of its molecular structure and physicochemical properties. The pigment is a water-soluble biliprotein whose molecule is a homodimer with monomers consisting of three major parts (Fig. 3) [53]: (i) the *N*-terminal photoreception domain (70 kDa); (ii) the flexible hinge region between the *N*- and *C*-terminal domains, and (iii) the *C*-terminal domain (55 kDa) responsible for dimerization and signal transduction. Of great importance for its properties is also the 10 kDa *N*-terminal extension (NTE) responsible for its activity [54], kinase properties and light stability [55], and differentiation into two native species (phyA′ and phyA″) [11, 56].

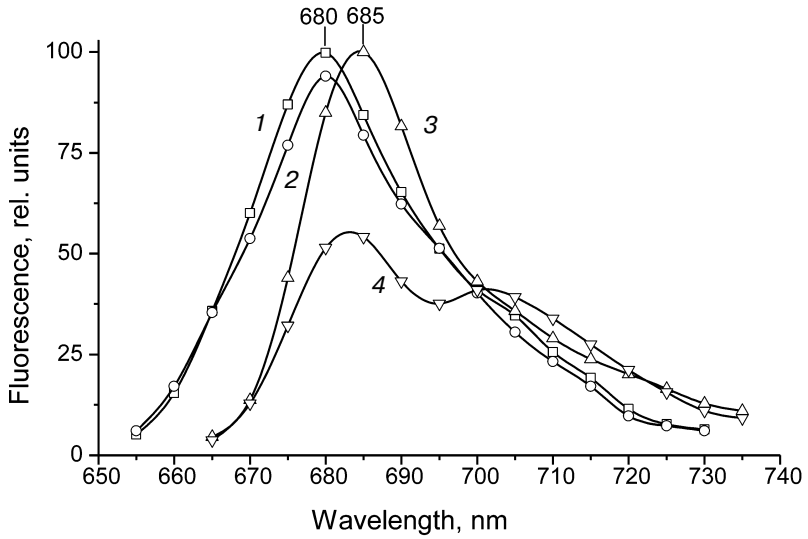

Fig. 4. Fluorescence emission spectra (85 K, λ_{ex} = 632.8 nm) of phytochrome in root tips (λ_{max} = 680 nm) (*1*, *2*) and coleoptiles tips (λ_{max} = 685 nm) (*3*, *4*) of etiolated wheat seedlings measured immediately after darkness when all the pigment is in the Pr state (*1*, *3*) and after saturating red illumination (λ_a = 632.8 nm) partially converting Pr into lumi-R, the first product stable at low temperatures (the state of photoequilibrium between Pr and lumi-R) (*2*, *4*). Note the difference in the position of the spectra (λ_{max}) and the extent of the photoconversion Pr → lumi-R (γ_1) measured as a relative decline of the intensity in the maximum, which are 680 nm and 0.05, respectively, for roots and 685 nm and 0.46 for coleoptiles. The spectra in the coleoptile and root tips represent, respectively, the two phyA native species, phyA′ and phyA″. The spectra were not corrected for the spectral sensitivity of the spectrofluorometer (adapted from [56]).

The pigment exists in two photointerconvertible states: the initial red light absorbing R state (Pr), in which it is synthesized and remains in the cytoplasm in the dark, and the physiologically active far red absorbing FR state (Pfr). Its action is based on the operation of the phytochrome cycle initiated by photoisomerization reactions of the chromophore in the Pr and Pfr states [57, 58]. Upon illumination, the activated photoreceptor in the Pfr form is transferred from the cytoplasm into the nucleus and operates there activating photoregulatable genes and thus promoting photomorphogenesis [59].

phyA is not homogeneous and is represented in etiolated tissues of mono- and dicots by two native species – phyA′ and phyA″ [11, 56]. phyA′ is the major and light-labile species, whereas phyA″ is minor, saturable by its content, and relatively light-stable. They differ in spectroscopic and photochemical properties with phyA′ and phyA″ being the longer- and the shorter-wavelength species, respectively, capable (Pr′) and incapable (Pr″) of the Pr-to-lumi-R conversions at cryogenic temperatures (Fig. 4). The later feature allowed their quantitative determination and characterization in plant tissues *in vivo* with the use of low-temperature fluorescence spectroscopy and photochemistry. It is important to mention that at ambient temperatures the extent of the Pr-to-Pfr conversion is essentially similar for both of them (0.80-0.85 for phyA′ vs. 0.75 for phyA″). Investigations of the phyA pools in transgenic plants and heterologous systems (*Pichia pastoris* and *Escherichia coli*) have shown that they are the products of one and the same gene and differ by the state of phosphorylation – phyA′ being phosphorylated and phyA″ dephosphorylated. Besides, phyA′ is water-soluble and phyA″ is membrane (protein)-bound. These phenomenological and physicochemical distinctions between the two phyA pools strongly imply their possible functional diversity (see below). Interestingly, phyB, in contrast to phyA, is present in plants as a single species similar in its phenomenological properties to phyA″.

The chain of events in the light signal transduction with participation of phyA includes the transfer of the photoreceptor in its Pfr form into the nucleus, which proceeds only after its association with the partner proteins FHY1 (far-red elongated hypocotyl 1) and FHL (FHY1-like) [59] (Fig. 5) because the nuclear localization signal (NLS) is lacking in phyA. In the nucleus, activation of the positive photomorphogenic gene factors (HY5, HYH, HFR1, CIP7, and LAF1) takes place via the suppression by Pfr of the negative regulation of the complex COP1/SPA (constitutive photomorphogenic 1/suppressor of *phyA-105*) and slowing of the E3–ubiquitin ligase machinery, which in the dark degrades transcription factors required for photomorphogenesis [60]. According to [61, 62], FHY1 may play a significant role in these processes by forming various phyA–FHY1 transcription factor complexes with HY5, PIF3, HFR1, and LAF1. Recent data show that a molecular mechanism for the inactivation of the COP1/SPA complex by phyA and also phyB includes direct interaction of their Pfr form with

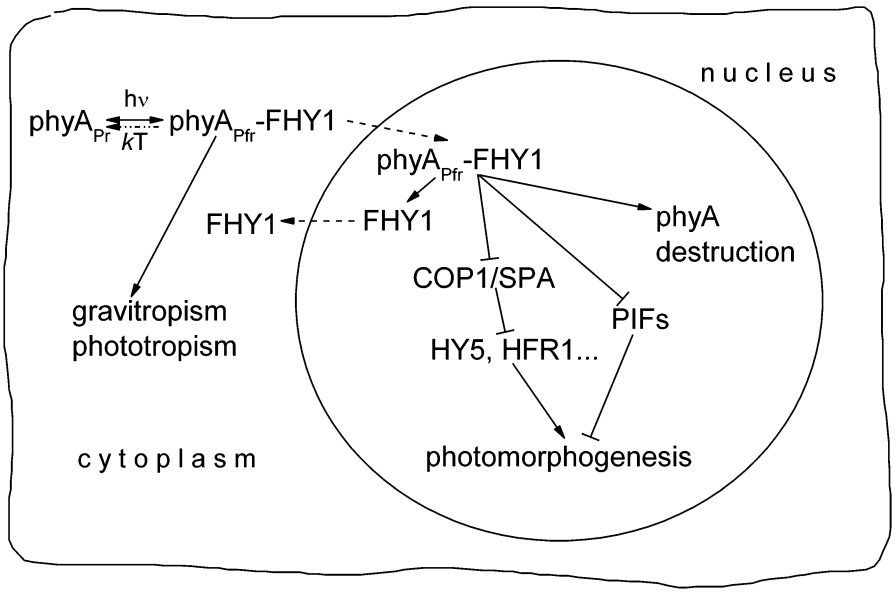

Fig. 5. Simplified scheme of the mechanism of action of phyA. phyA in its Pr state upon illumination undergoes photoisomerization converting it into the Pfr state. This is followed by its association with a partner protein FHY1 (or its analog FHL) and their transfer into the nucleus. Pfr–FHY1 interferes there with COP1/SPA, deactivating it, and forms complexes with transcription factors (HY5, PIF3, HFR1, and LAF1), which in the dark are destroyed by COP1/SPA but are accumulated in the light. These complexes regulate the expression of a broad range of genes. Another branch of Pfr signaling includes destruction of PIFs that interfere with expression of light-regulated genes in darkness. In the cytoplasm, phyA in the Pfr form modifies photo- and gravitropism and mediates fast biochemical processes.

SPA1 and other SPA proteins leading to their disruption and reorganization [60]. On the other hand, phyA inhibits negatively-acting PIFs (PIF1, PIF3, PIF4, and PIF5) [63] and related proteins HFR1 and PIL1 [64] via their phosphorylation followed by ubiquitination and degradation [48]. Activation by phyA of the positive transcription factors via these two processes results in the expression of over a thousand genes participating in photomorphogenesis (see above and [3, 16]).

The most striking functional distinction of phyA from the other phytochroms is, however, its responsibility for mediation of the atypical irreversible very low fluence responses (VLFR) and high irradiance responses (HIR) initiated by FR light. The VLFR can be induced by brief pulses of light and saturate at a very low relative Pfr content ($\leqslant$0.1%). And to reach a HIR effect, constant or relatively frequent and high intensity light is required [3]. The maximum in the action spectrum of the HIR is located typically between the absorption maxima of the Pr and Pfr forms (around 710 nm), the region optimal to reach maximum Pfr concentration in the nucleus in the process of the phyA shuttle from the cytoplasm to the nucleus with the help of FHY1 and FHL [65]. Along with these photoresponse modes, phyA performs also the classical R-induced FR-reversible low fluence responses (LFR), which are characteristic for phyB [9, 66].

Manifestation of the VLFR and HIR depends on the state of phosphorylation of the pigment at the NTE as revealed by investigations of the deletion and serine-to-alanine (Ser/Ala) substitution of phyA mutants of tobacco, rice, and *Arabidopsis* expressed in transgenic *Arabidopsis* [66-68]. Since the Ser/Ala substitution prevents phosphorylation of the phyA molecule at the NTE, a role for phosphorylation discriminating between the different phyA-dependent responses was proposed [66]. Mutation in the PAS2 domain (Glu777Lys) prevents HIR but does not affect VLFR, suggesting that this molecular region also participates in determining the functional specificity of phyA [69]. The differences between the photoresponse modes are localized downstream of the light-signal transition chain as well [3]. phyA is thus capable of transducing the light stimulus along the two different pathways. In our experiments, it was shown, on the other hand, that these modifications in the NTE and domination because of that of either of the response modes correlated with disappearance of one of the two native phyA species, and the VLFR was assigned to phyA′, whereas HIR – to phyA″ [11, 12]. We thus have to take into consideration this functional heterogeneity of phyA in our analysis of photoregulation of Chl biosynthesis and formation of the photosynthetic apparatus.

phyA-mediated regulation of Chl biosynthesis. Investigation of the regulation by phytochrome of Chl biosynthesis meets with the problem of separation of the effects resulting from the light-induced $Pchlide^{655}/_{650}$ conversion into Chlide from those induced by activation of phytochrome. The methodology to overcome this difficulty comprises three major approaches: first, the employment of the traditional for phytochrome photophysiology R/FR reversibility of the light effects; second, the use of phytochrome mutants and transduction chain mutants; and third, experiments under FR light inactive in the Pchlide $\rightarrow$ Chlide conversion but efficient in phyA activation.

The first key regulatory step in Chl biosynthesis is at the formation of ALA (see above and Fig. 2a). Upon transition of seedlings from darkness to light, upregulation of ALA accumulation was detected [70, 71]. Phytochrome was implicated in the light regulation of Chl accumulation at this point [72]. Later on, Terry and coauthors [42, 73, 74] followed coordinated synthesis of Chl and the Chl *a*/*b*-binding proteins and showed that *HEMA1* and *Lhcb* expression are regulated by phytochromes acting in HIR and LFR modes (Fig. 2b). In recent observations, Tang et al. [75] identified two transcription factors – FHY3 (far-red elongated hypocotyl 3) and FAR1 (far-red impaired response 1) – as positive regulators of Chl biosynthesis in *Arabidopsis*. FHY3 directly binds to the promoter and activates expression of *HEMB1*, which encodes δ-aminolevulinic acid dehydratase (ALAD) in the Chl biosynthetic pathway. FHY3 physically interacts with the negative transcription regulator PIF1 to coordinate Pchlide synthesis and seedling greening.

Another key regulatory point is at the end product of its dark stage – on the formation of complex Pchlide–POR–NADPH (Fig. 2a). It is well documented that phytochrome mediates a decrease in the content of *POR* mRNA during illumination of etiolated seedlings [76]. Later on, two POR isoforms, PORA and PORB, were detected in barley [77], one of which (PORA) is downregulated by light and the other (PORB) is relatively light-independent. The third protein, PORC, was found in *Arabidopsis* ([78, 79] and the literature cited therein) in green tissues, which revealed positive dependence on the intensity of the actinic light. The number of *POR* genes thus varies in different plant species, although some plants contain only one *POR* gene (see references in the monograph of Belyaeva [33]). Under a given light regime, plants may use preferentially one of the three enzymes to keep the optimal level of Chl synthesis.

After the Pchlide-to-Chlide photoconversion, a number of consecutive reactions takes place connected with alterations of the pigment–protein complex and the esterification of Pchlide and Chlide formation (see above and Fig. 1). The rate of Chlide esterification [80, 81] and of the "Shibata shift" [82] can be increased by a R pulse pretreatment of etiolated seedlings, the effect being reversed by a FR pulse. The extent of the effect was different in both cases, and the authors thus came to the conclusion that phytochrome (most likely phyB because of its R-induced FR-reversed character, although phyA cannot be excluded) independently controls the Chlide

esterification and the "Shibata shift". It was also shown that phytochrome (i) significantly stimulated regeneration of the feedback inhibited Pchlide [72, 83] and (ii) enhanced the rate of formation of the apoproteins and a number of receptor sites for chlorophylls [84] (Fig. 2b). It should be noted that the expression of nuclear genes encoding various chloroplast proteins might be feedback regulated by the level of Chl or Chl precursors [85].

Mutants with violations in the regulation of plant development in darkness (scotomorphogenesis) resulting in partial transitions to light development (photomorphogenesis) were particularly instrumental in unraveling the mechanism of signal transduction from phytochromes (reviewed in [86, 87]). These mutants (*cop*, *det*, *fus*, *lip*) have impairments in the system of suppression (comprising the COP1/SPA complex) of the photosynthetic genes. Their respective wild-type gene products act to repress the photomorphogenic type of development in the dark including tetrapyrrole accumulation. Sperling et al. [88] investigated such a mutant (*Arabidopsis cop1-18*, previously *det340*) for elucidating the roles of PORA and PORB in prolamellar body (PLB) formation and Pchlide$^{655}/_{650}$ assembly during scotomorphogenesis and showed that the two *POR* genes are interchangeable in these processes. In the context of the present discussion, of interest is a pea mutant with light-independent photomorphogenesis (*lip1*) [89], because peas possess a less complicated system of phytochromes (only phyA and phyB) [90] and of PORs (one species) [91]. The *lip1* mutant has agranal plastids with thylakoids (unlike wild-type seedlings with etioplasts and PLBs), dominating Pchlide$^{633}/_{628}$ and POR [26, 89]. At the same time, Pchlide$^{655}/_{650}$ is repressed and PLBs are not formed. Seyyedi et al. [26] concluded that the accumulation of POR (in pea) is insufficient to induce formation of PLBs and Pchlide$^{655}/_{650}$ and that some other factors are needed for this. The mutant was also characterized [89, 92] by the state of phyA. Its content declined from 3- to 10-fold depending on the tissues/organ and age of the mutants without violation of the phyA′/phyA″ proportion. These effects were considered as the result of the activation of the phytochrome signal transduction chain in darkness in the mutant. Of interest is the fact that the *lip1* mutation did not affect the level of phytochrome in roots, suggesting tissues/organ specificity in the regulation of *phyA* gene expression [92].

Further genetic analysis of the phytochrome signal transduction mutants implicated PIFs as negative regulators of Chl biosynthesis. PIF1 was shown to negatively regulate key genes in the Chl biosynthetic pathway in the dark and seed germination in FR light [16, 48, 93]. The level of Pchlide and POR in the dark is also controlled by PIFs, and this repressive effect of PIFs is reversed by light via proteolysis of the PIFs [16]. PIF1 was shown to play a similar role in carotenoid synthesis (coordinated with Chl biosynthesis and chloroplast development), suppressing in darkness the gene encoding phytoene synthase (PSY), the main rate-determining enzyme of the pathway and promoting it upon illumination [94].

Specificity of Chl biosynthesis and its regulation by phyA under FR-enriched light. The photophysiology of plants living under deep canopy shade and actinic light with dominating FR spectrum is to be distinguished by at least three essential features: (i) slowed Chl accumulation, (ii) imbalance between the two photosynthetic systems with domination of PSI, and (iii) domination of phyA as a photoreceptor. Nevertheless, the higher plants have overcome these difficulties through coordinated action of a number of key factors optimizing plant physiology even under these conditions. However, under laboratory conditions with strict FR illumination (constant or pulsed with $\lambda_a \geqslant 700$-720 nm), Pchlide-to-Chlide phototransition is not possible and the effect of phyA can be investigated *per se* [4, 5, 88]. phyA mutants (*fri* mutants of tomato) turned out to be completely insensitive to FR light inhibition of hypocotyl growth [4]. Seedlings grown in darkness of both *fri* mutants and the wild type became green on transfer to white light. However, the wild-type seedlings grown in FR lost their capacity to green in white light, while the *fri* mutants de-etiolated normally.

A similar picture was observed by Barnes et al. [5] with phyA mutants and transduction chain components downstream from phyA (*fhy1*) of *Arabidopsis*, suggesting that the phyA-dependent mechanisms that regulate this process may be conserved between species. It was also found that phyA after FR illumination not only inhibited Chl accumulation upon following R and W (white) illumination, but they became lethal after this treatment [4, 5]. In pea plants exposed to continuous FR, plastid RNA and DNA accumulations were enhanced [95]. phyA-mediated repression of the *POR* gene and also the accumulation of Rubisco large subunits under FR were demonstrated in tobacco plants (*Nicotiana tabacum* L.) [96]. These FR-stimulated responses belong to the phyA-mediated HIR mode [97].

All this implies that the inhibition of Chl biosynthesis under R and W light is the consequence of the phyA regulatory activity in seedlings during their growth under FR light. Barnes et al. [5] interpreted this effect as a consequence of severe repression of the *POR* genes by FR light coupled with irreversible plastid damage leading to the separation of the normally coordinated phytochrome-mediated *POR* repression and light-dependent Pchlide reduction. Indeed, wild-type *Arabidopsis* seedlings grown under continuous FR (FRc) [5, 98, 99] and the dark-grown *det340* photomorphogenic mutant seedlings [100] lack PORA, *PORA* mRNA, photoactive Pchlide$^{655}/_{650}$ and PLBs, and they fail to green normally during subsequent exposure to W light. At the same time, *Arabidopsis PORA* or *PORB* overexpression overcomes the FRc-induced block in greening caused by photooxidative damage to plastids in white light [88]. Thus, the block of greening is

explained by the lack of the products of the *POR* genes expression and of Pchlide$^{655}/_{650}$ and by the overaccumulation of free molecules of Pchlide$^{633}/_{628}$, a potent sensitizer for photooxidative damage [5, 88, 98, 99, 101].

Working along this experimental line, McCormac and Terry [74] examined the expression of *HEMA1* during the phyA-mediated FR block of greening under W light in *Arabidopsis*. This effect comprised two separate responses: one was caused by a loss of *HEMA1* and *Lhcb* expression (transcriptionally coupled response) and the other was a transcriptionally uncoupled response. The latter correlated with a deregulation of tetrapyrrole synthesis and photooxidative damage. Both effects led to the loss of nuclear gene expression and were inhibited by overexpression of *POR*. Based on that, the authors suggested a role for plastid signaling in the FR-mediated pathway. Further investigations [42] have shown that feedback mechanisms from functional plastids are required for normal chloroplast development. The authors followed the expression of three nuclear genes – *Lhcb*, *HEMA1*, and *GSA* – under FR pre-treatment and showed that *Lhcb* was most strongly repressed and that protein GUN1 was dominant for the plastid feedback regulation of *HEMA1*. Interestingly, Terry and coworkers [102] recently advanced a new model in which a singlet oxygen retrograde signal, generated by chlorophyll precursors, inhibits expression of key photosynthetic and Chl synthesis genes to prevent photooxidative damage during de-etiolation.

Speaking of the Pchlide-to-Chlide photoconversion under FR enriched conditions, it should be mentioned that these light conditions correspond to the specific type of photoconversion that is carried out under low light intensity (see above and Fig. 1). There is a side way of Chl formation – appearance of the short-wavelength form Chl$^{675}/_{670}$, which becomes the major one at low light intensity [103]. This should correspond to the conditions of plants growth under FR light enriched conditions with relatively low input of photochemically active R light at around 650 nm (for instance, under the shade of thick foliage). Under these conditions, this branch of the Pchlide conversion may become the major one, which needs experimental verification.

Alterations of the sign of phyA regulation of Chl biosynthesis. Functional interaction of phyA and hormones. As discussed above, FR brings about suppression of POR and Pchlide$^{655}/_{650}$ accumulation, destruction of PLBs, and inhibition of the following greening under W light, effects belonging primarily to FR-HIR. However, this may not be a universal phenomenon: it is well documented in monocots [76, 104]; in dicots, it has been less clearly defined [105, 106]. According to Meyer et al. [7], the suggested inverse regulation by light of Chl formation and POR activity is not generally acceptable for all the higher plants. The diverse effects of light on *POR* in different plant species were interpreted as superposition of the down-regulation of *PORA* and the constitutive expression of *PORB* [77]. However, the situation may be more complex than that. This hypothesis may not explain, in particular, the complex dynamics of POR – negative light effect was overcome by positive in developing leaves of peas [107] containing only one POR [91].

In our experiments, the sign and extent of the effect of FRc on Pchlide$^{655}/_{650}$ accumulation were also shown to depend on plant species and its organs/tissues [8]. In the cotyledons of tomato and *Arabidopsis* grown under FRc, Pchlide$^{655}/_{650}$ decline was observed, in agreement with the data of Runge et al. [98] and Barnes et al. [5] (Fig. 6). These effects obtained primarily with dicotyledonous plants were supported by experiments on monocot rice (*Oryza sativa* L. cv. Nipponbare) and its mutants deficient in phyA, phyB, or phyA and phyB [108]. FRc brought about a steep decline of both Pchlide$^{633}/_{628}$ and Pchlide$^{655}/_{650}$ in the wild-type (WT) plant and also its *phyB* mutant, shortening of the coleoptiles and appearance of the first leaf, whereas pulsed FR (FRp) was of low effectiveness. The latter allowed us to attribute these responses to HIR, in agreement with [69]. However, in tobacco cotyledons and pea leaves, and also in the upper parts of stems of all the investigated plants, i.e., tobacco, pea, tomato, and *Arabidopsis*, a positive effect of FRc on Pchlide$^{655}/_{650}$ accumulation of different magnitude was observed (Fig. 6). It should also be mentioned that the different signs of the FRc effect on Pchlide$^{655}/_{650}$ are not connected with the availability of the Pchlide chromophore [109].

These variations of FRc effects are reminiscent of the light regulation of *phyA* mRNA [110]. The authors found three *PHYA* transcripts, which are differently regulated depending on their localization in the plant. This was interpreted as a manifestation of the fact that the *PHYA* gene is subject to the regulation of multiple signals (from the environment, those connected with plant development, and organ/tissue-specific signals). Existence of similar properties in the case of the *POR* gene can be assumed. Armstrong et al. [77] followed also an analogy between PORA downregulated by light and constitutively expressed PORB with light-regulated phyA and relatively light-stable phyB. There seems to be a common mechanism distinguishing between the two modes of light regulation. Thus, we may conclude that the sign of the phyA effect on *POR* gene expression (i.e., induction or repression) and its extent are modulated by signals specific with regard to the plant species and organs/tissues.

Of interest is an observation on the mutant of rice deficient in phyA [108]. [Pchlide$^{633}/_{628}$] and [Pchlide$^{655}/_{650}$] were the same in the WT and the mutant in the dark and did not change under FRp. However, under FRc [Pchlide$^{655}/_{650}$] increased by about 100%, which points to possible participation of phyC in this process (via HIR), albeit inducing expression instead of inhibiting expression as seen for phyA. This agrees with the data of Takano et al. [9, 111], who observed positive regulatory action of

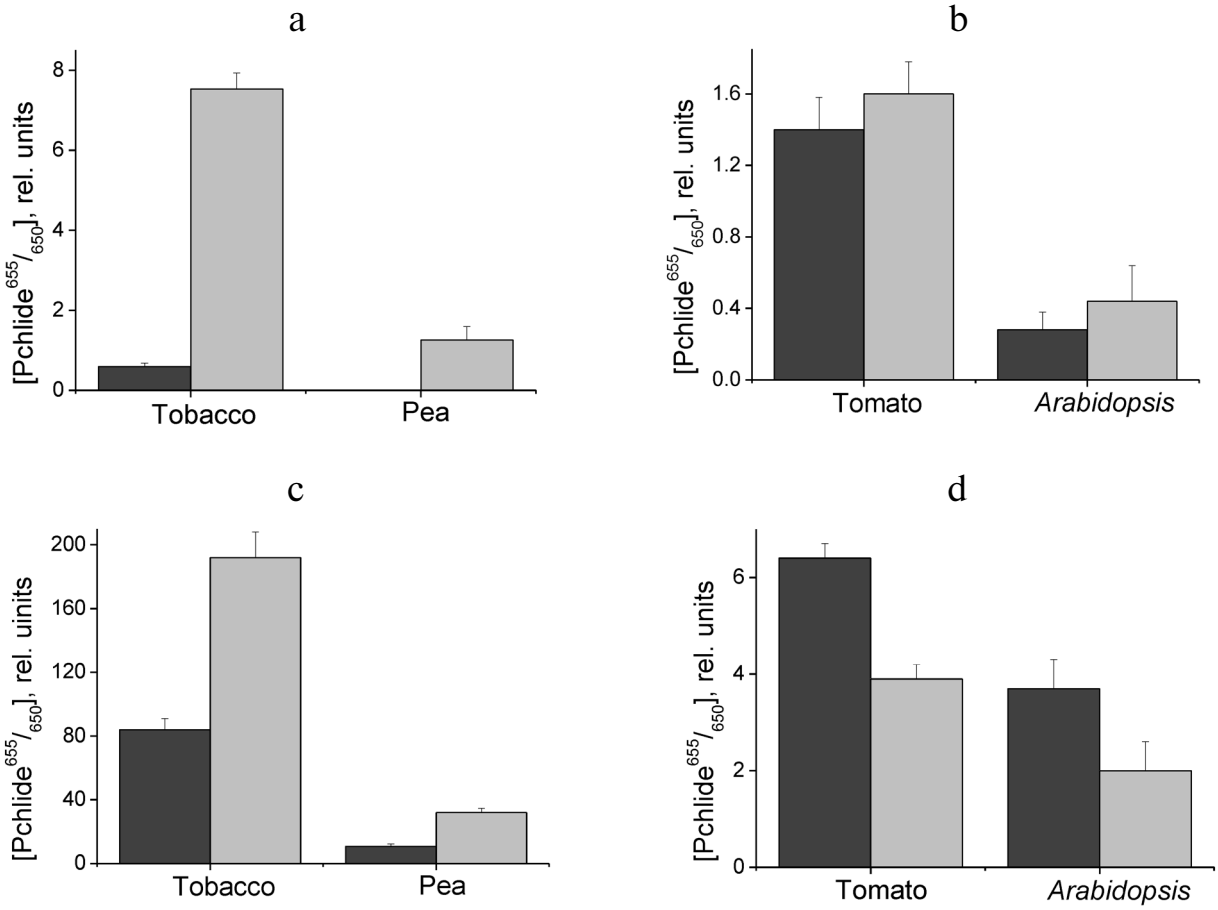

Fig. 6. Content of active Pchlide$^{655}/_{650}$ in etiolated (left black columns) and FR-grown (right gray columns) seedlings of tobacco, pea, tomato, and *Arabidopsis*: a, b) hypocotyls; c, d) cotyledons of tobacco, tomato, and *Arabidopsis* and primary leaves of pea (adapted from [8]).

FRc on the light-dependent genes coding for the Chl *a*/*b*-binding protein (CAB) in the *phyA*-deficient mutant of rice and the absence of it in the double mutant *phyAphyC*. Thus, the sign of the effects of FRc on the active Pchlide biosynthesis depends also on the photoreceptor – phyA or phyC in this case.

In current phytochrome research, the interplay between light and other signals is becoming one of the central issues (see reviews [15, 87, 112, 113]). In particular, hormones are closely implicated in phyA signaling determining the sign of light effects on Chl biosynthesis. Kobayashi et al. [18] observed opposing auxin/cytokinin effects on light regulation of Chl accumulation in *Arabidopsis* roots – repression by auxin and promotion by cytokinin. In experiments by Roy et al. [114], phytochrome induced suppression of Chl biosynthesis in etiolated (rice) leaves upon high intensity R illumination of the shoot bottom, which resulted from reduced ALA accumulation. They attributed the effect to phyA and R-induced HIR. Possible participation of hormones could explain the distant character of the effect. Cheminant et al. [51] demonstrated a crucial role of gibberellin-regulated DELLA proteins in the formation of functional chloroplasts during de-etiolation. These proteins regulate the levels of POR, Pchlide, and carotenoids in the dark by repressing PIFs. Hormone ethylene regulation integrates with that of phytochrome via co-action of PIFs and the ethylene signaling transcriptional factors EIN3/EIL1. Ethylene induces *PORA* and *PORB* gene expression, represses the accumulation of Pchlide, and in general enhances seedling greening [115]. It activates also PIF3 via EIN3, resulting in stimulation of plant growth in the light and its inhibition in the dark. Light thus reverts the sign of the ethylene action [113].

In our experiments, the connection between the phyA signaling and hormone action was demonstrated by reversion of the sign of the phyA effect on Pchlide$^{655}/_{650}$ accumulation [10] in the rice (*Oryza sativa* L. Japonica cv. Nihonmasari) mutant *hebiba* deficient in the hormone jasmonic acid (JA) involved in response to biotic and abiotic stresses (such as wounding and defense) [116]. In darkness, this mutant had long mesocotyls and short coleoptiles, whereas the situation was reversed under FRc (λ_a > 720 nm) – short mesocotyls and long coleoptiles.

This proves the participation of phyA in these light effects, albeit with the sign opposite to that observed in the wild-type rice – short coleoptiles and even the appearance of the first leaf [10, 116]. In the dark, phyA′/phyA″ content was the same in WT and *hebiba*, suggesting that JA in darkness does not affect the rate of phyA synthesis and its differentiation into the subpools. Under FRc, [phyA] dropped down in WT and the phyA′/phyA″ balance shifted towards phyA″. However, in *hebiba* the light-induced phyA decline was less pronounced and the equilibrium between phyA′ and phyA″ was not affected. This indicates that JA negatively interferes with the action of phyA under FRc (HIR), implicating it in the regulation of light-induced phyA turnover [10, 117].

In the case of Pchlide biosynthesis in rice, the role of JA in the phyA signaling was even more profound [10]. The total Pchlide$^{633}/_{628}$ and Pchlide$^{655}/_{650}$ contents were higher in the mutant, which implies that JA suppresses its synthesis in the wild type. However, the most pronounced effect of JA on phyA signaling was observed in the case of Pchlide$^{655}/_{650}$ biosynthesis. In the WT, pulsed FR (FRp) stimulated biosynthesis of the pigment, whereas FRc of the same dose was inhibiting (Fig. 7). In contrast, both FRp and FRc stimulated Pchlide$^{655}/_{650}$ biosynthesis in the mutant, i.e., the sign of the FRc effect changed from negative in the WT to positive in *hebiba*. Taking into account the fact that HIR is attributed to phyA″ and VLFR to phyA′ in *Arabidopsis* [11, 12], we speculate that the FRc and FRp effects on Pchlide$^{655}/_{650}$ accumulation in wild-type and *hebiba* rice are mediated by phyA″ and phyA′, respectively. These observations agree with the data showing that JA suppresses *PORA* in seedlings grown in darkness [118]. Also, block of the greening response by FR light is at least in part regulated by JA [118].

In general, JA and phytochrome signals are mutually antagonistic (for review, see [15]). JA biosynthesis genes are induced by phyA; however, a negative feedback loop exists in which JA signaling is inhibited by phytochrome signaling. On the other hand, JA triggers the phosphorylation of phyA [119], possibly, at Ser598 [15], which downregulates activity of the photoreceptor via suppression of its interaction with transduction chain partners [120]. phyA activity is also downregulated by JA via promotion of its degradation [10, 117].

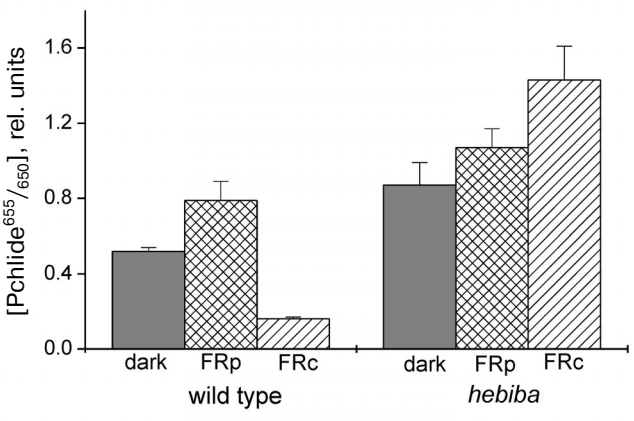

Fig. 7. Content of active Pchlide$^{655}/_{650}$ in coleoptile tips of wild-type rice and its *hebiba* mutant. The seedlings were grown for 5 days in darkness or under pulsed (FRp) or continuous (FRc) FR ($\lambda_a \geqslant 720$ nm) of equal total fluence (adapted from [10]).

Gibberellin and abscisic acid are also involved in the phytochrome signaling. Complex regulatory loops between light, gibberellin, and abscisic acid signaling pathways were revealed [121]. Luccioni et al. [122] observed suppression of HIR and LFR in the brassinosteroid (BS) biosynthesis of *Arabidopsis* mutant (*eve1*), suggesting implication of BSs as positive factors in the phyA signaling. It is becoming more and more clear that the key light signaling components (PIFs and HY5 and others) serve as a link of light signals to the signaling of phytohormones in regulating seedling photomorphogenesis and seed germination (see reviews [16, 87, 112]).

The above effects of JA, BSs, and other hormones modifying phyA signaling can be thus considered as yet another way to fine-tune the phytochrome system and help to adjust the plant to changing environmental light conditions.

Fine-tuning of phyA action. Regulation of phyA′/phyA″ balance. Regulation of phyA activity includes the well-known phenomenon of its destruction and suppression of its biosynthesis upon illumination. This adjustment of phyA functioning to the environmental light conditions may be even more complex given the existence of the two native species of the pigment with different modes of action (for review see [11, 56]). Our investigations revealed four different effects of light on their content and equilibrium. First, R light induced destruction of phyA, primarily in its light-labile phyA′ form. Second, down-regulation of phyA synthesis under FR light without violation of the phyA′/phyA″ balance. Third, in etiolated seedlings (of *Arabidopsis*), the phyA′/phyA″ balance is regulated by germination-inducing W light pre-illumination shifting it towards the light-labile phyA′ [11, 12]. And fourth, conversion of phyA′ into phyA″ upon R illumination of etiolated (barley) coleoptiles preceding phyA destruction (V. Sineshchekov and L. Koppel, unpublished results). On the other hand, the organ/tissue- and development-specific phyA signaling may also be realized via variations in the content of the two phyA species in the plant. In this connection, the fact is of interest that the phyA′/phyA″ balance depends on two major regulatory factors in the cell, i.e., the phosphatase/kinase equilibrium in the cytoplasm and its pH [123].

It should be noted that phosphorylation of phyA at the NTE supposedly converting phyA″ into phyA′ [11, 12] does not involve the known autophosphorylation of serine-8 and serine-18 (in oat phyA) [124], which makes phyA more light-labile and less functionally active [55]. The ser-

ine phosphorylation at the NTE may thus help to achieve two goals – differentiation of phyA into phyA″ and phyA′ (acting, respectively, in HIR and VLFR) and modification of the phyA sensitivity by changing its lability to light.

These light effects on the phyA′/phyA″ content may intimately relate to the phyA regulation of Chl biosynthesis because both VLFR and HIR were shown to be implicated in this process. According to Kneissl et al. [66], wild-type rice phyA and mutant rice phyA SA (with substitution of 10 serines to alanines at the NTE) expressed in transgenic *Arabidopsis* lines complemented the wild-type phenotype in preventing Chl accumulation in seedlings grown under constant FR (the HIR conditions) (FR block of greening, "far-red light killing effect"). Under pulsed FR (the VLFR conditions), the phyA SA mutant was less efficient than the wild-type phyA in the far-red killing effect. Since the mutant rice phyA SA has phyA″, whereas the wild-type rice phyA has both phyA′ and phyA″ [12], we may conclude that phyA″ is a dominating species participating in these FRc-induced Chl biosynthesis inhibition effects (the HIR mode). This is in general in line with the attribution of the HIRs to phyA″ and VLFR to phyA′ [11, 12]. The observation by Kneissl et al. [66] that expression of phyA SA led to a hypersensitive effect under FRc and no strong inhibition of hypocotyl elongation under R light, whereas the wild-type rice phyA-expressing lines were hypersensitive under R light, not however under FRc, supports this conclusion. Also, in favor of this conclusion is the observation that under VLFR conditions (FRp), the inhibition of hypocotyl elongation was stronger in the lines expressing the wild-type rice phyA than the phyA SA. Thus, experimental evidence is being accumulated that underscores the necessity to take into account the structural and functional heterogeneity of phyA in the analysis of the light regulation of Chl biosynthesis and formation of the photosynthetic apparatus.

Transition from the scoto- to photomorphogenic ways of life is accomplished in plants by the two major light-induced events – photoconversion of Pchlide to Chlide and further on into Chl and photoactivation of light-inducible genes coding for proteins of the photosynthetic apparatus. During scotomorphogenesis, the signaling systems suppress tetrapyrrole gene expression in order to avoid their potentially harmful excessive accumulation and stimulate expression of the genes of the enzymes PORA and PORB, which bind photodestructive free Pchlide. Upon illumination, the signaling systems promote accumulation of (proto)chlorophyll(ides) and their binding proteins. This is achieved primarily by the action of phyA, which is responsible for the regulation of more than one thousand early responding genes, including those regulating Chl biosynthesis.

The physicochemical mechanisms of the Pchlide → Chlide and Pr → Pfr conversions are becoming more and more clear, as well as the steps both in the Pchlide conversion into Chl and in the phyA signal transduction cascade. At the same time, the whole net of photoreceptors acquiring and transducing environmental signals and interacting with the inner regulation systems (in particular, hormonal) reveals its extremely complex character. The dynamics, extent, and mode of action (VLFR, LFR, and HIR) and even the sign of the photoresponses with participation of phyA strongly depend on the plant/organ/tissue specificity of the signal and on its connection with the stage of plant development. In the other words, they strongly depend on the cellular context of the plant under investigation, including its genetic background. The specificity and complexity of the phyA action are connected with the fact that it is the major photoreceptor in higher plants mediating both R and FR signaling, which is vitally important for plant survival especially under conditions of dense canopy. The photoreceptor also possesses an extremely and exceptionally high sensitivity of light perception, which is important for initiating seed germination and photomorphogenesis. Along with the plant development, this extreme sensitivity of phyA, which may become harmful to the plant because of overstimulation of its de-etiolation (Pchlide accumulation, in particular), is downregulated at the levels of phyA biosynthesis, its destruction, and the intensity of signaling processes downstream of phyA.

Further complexity of this picture is added by the fact that phyA is represented in plants by its two native species, phyA′ and phyA″. They differ in spectroscopic and photochemical properties, association with membrane (protein), light-stability, and, most importantly, by the mode of action – VLFR in the case of phyA′ and HIR and, possibly, LFR, in the case of phyA″. Their total content and proportion and thus activity change depending on the plant species, organ and tissue, its stage of development, illumination conditions, and other factors. Regulation of the phyA′/phyA″ abundance is yet another facet of the highly efficient mechanism of phyA fine-tuning. Of importance in this connection is phosphorylation of phyA as a means to provide phyA differentiation into its two native species, phyA′ and phyA″, and modulate light-lability and its kinase activity towards its interacting partners (PIFs, FHY1) downregulating its activity. All this is applicable to the issue of phyA regulating Chl biosynthesis and development of the photosynthetic apparatus, a process which strongly depends on the crosstalk of light and hormonal signaling. An encouraging sign in unraveling this complex situation is the finding that there exists in plants a central regulation knot – the phytochrome-interacting factors, PIFs, closely cooperating with the families of the hormonal and other transcription factors (EIN3/EIL1 and others). This promises that our knowledge of the light, hormonal, and other regulatory factors in plants can be structured around such integrating points.

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Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval

This article does not include research involving the participation of people or animals.

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