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Review

Research review

Phytochrome B phosphorylation expanded: site-specific kinases are identified

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Summary

The phytochrome B (phyB) photoreceptor is a key participant in red and far-red light sensing, playing a dominant role in many developmental and growth responses throughout the whole life of plants. Accordingly, phyB governs diverse signaling pathways, and although our knowledge about these pathways is constantly expanding, our view about their fine-tuning is still rudimentary. Phosphorylation of phyB is one of the relevant regulatory mechanisms, and – despite the expansion of the available methodology – it is still not easy to examine. Phosphorylated phytochromes have been detected using various techniques for decades, but the first phosphorylated phyB residues were only identified in 2013. Since then, concentrated attention has been turned toward the functional role of post-translational modifications in phyB signaling. Very recently in 2023, the first kinases that phosphorylate phyB were identified. These discoveries opened up new research avenues, especially by connecting diverse environmental impacts to light signaling and helping to explain some long-term unsolved problems such as the co-action of Ca^{2+} and phyB signaling. This review summarizes our recent views about the roles of the identified phosphorylated phyB residues, what we know about the enzymes that modulate the phospho-state of phyB, and how these recent discoveries impact future investigations.

Introduction

Energy-providing light is a critical environmental factor for sessile plants; therefore, they have to optimize their growth and development to the ever-changing light conditions. To achieve this, they have evolved light-sensitive photoreceptor molecules. Phytochromes (*PHY*) are the receptors of red (R, λ_{max} *c.* 660 nm) and far-red (FR, λ_{max} *c.* 730 nm) light. *Arabidopsis thaliana* possesses five phytochromes named phyA through phyE (Sharrock & Quail, 1989; Li *et al.*, 2015). phyA is the dominant receptor of extremely dim light and high-irradiance FR light, whereas phytochrome B (phyB) is the dominant mediator of classical R/FR responses (Bae & Choi, 2008).

Phytochromes work as molecular switches: they are synthesized in their inactive (Pr) form, and after perceiving red light irradiation they are converted to the biologically active Pfr conformer that governs a complex signaling network. FR irradiation converts Pfr back to Pr, inactivating the signaling. Additionally, Pfr is thermodynamically unstable and converted spontaneously to Pr, a process called thermal reversion. This phenomenon is masked by the production of fresh Pfr molecules under strong red light, but can be a dominant factor in reducing the available active Pfr pool under dim light conditions or in the dark (Klose *et al.*, 2020).

Phytochromes are functional in dimers; each monomer is *c.* 125 kDa and consists of an N-terminal photosensory module (PSM) and a C-terminal output module (OPM) connected by a flexible hinge region. The PSM has a linear tetrapyrrol chromophore attached and, thus, is responsible for the light sensitivity and conformation changes of the molecule. It contains an NTE (N-terminal extension), a PAS (Per/Arnt/Sim), a GAF (a cyclic guanosine monophosphate phosphodiesterase/adenylyl cyclase/FhlA) and a PHY (phytochrome-specific) domain (Rockwell *et al.*, 2006; Burgie *et al.*, 2014). The OPM is required for phytochrome dimerization and contains two PAS domains and a HKRD (Histidine Kinase Related Domain; Fig. 1a; Nagatani, 2010; Qiu *et al.*, 2017; Li *et al.*, 2022).

The nuclear import of phytochrome Pfr and its localization to nuclear molecule complexes (termed photobodies, PBs) are an

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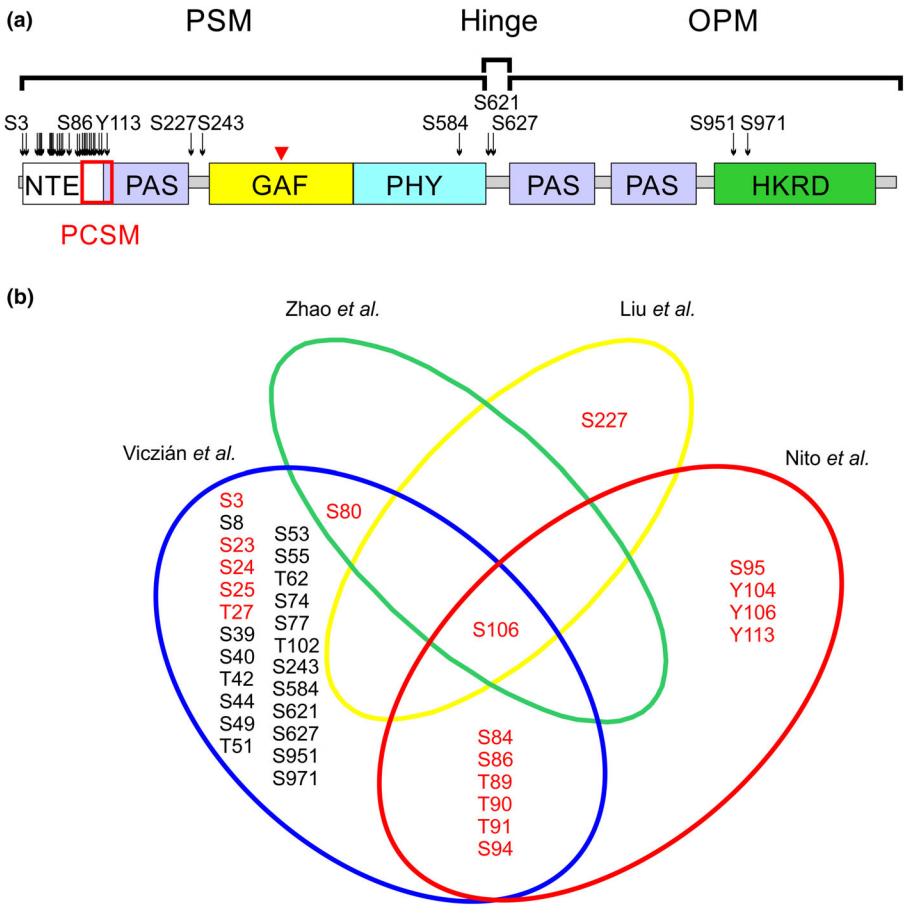

Fig. 1 Domain structure of Arabidopsis phytochrome B (phyB) and location of phosphorylated amino acids. (a) Schematic representation shows the domain structure of a phyB monomer. Arrows indicate *in vivo* identified phosphorylated amino acids (Medzihradszky *et al*., 2013; Nito *et al*., 2013; Viczián *et al*., 2020; Liu *et al*., 2023; Zhao *et al*., 2023). GAF, cyclic guanosine monophosphate phosphodiesterase/adenylyl cyclase/FhlA domain; HKRD, Histidine Kinase Related Domain; NTE, N-terminal extension; OPM, C-terminal output module; PAS, Per/Arnt/Sim domain; PCSM, phosphorylation cluster of signaling modulation; PHY, phytochrome domain; PSM, N-terminal photosensory module. The red triangle indicates the chromophore that is covalently attached to Cystein 357. (b) Phosphorylated amino acids grouped according to the works in which they were described (Nito *et al*., 2013; Viczián *et al*., 2020; Liu *et al*., 2023; Zhao *et al*., 2023). Medzihradszky *et al*. (2013) confirmed and examined the phosphorylation of S86 for the first time, but this work is not marked here as an additional subset to simplify the figure. Red fonts depict those amino acids that have been tested for function in light signaling.

essential part of the signaling. Although the PBs' functions are not yet clarified, it has been shown that phyB interacts inside them with a set of proteins (Pardi & Nusinow, 2021; Kim *et al*., 2023) including PHYTOCHROME INTERACTING FACTORs (PIFs). PHYTOCHROME INTERACTING FACTORs are transcription factors; they act as a signaling hub connecting light, temperature and hormonal signaling, and their co-degradation with phyB Pfr is a key early light signaling step after the onset of light (Leivar & Quail, 2011; Ni *et al*., 2014).

Light signaling is modulated by post-translational modifications (PTMs) of phytochromes. Phosphorylation is one of these and has been examined for decades. Most of the early studies focused on monocot phyA due to its easy accessibility (purification, expression, etc.; Hunt & Pratt, 1980; Wong *et al*., 1986; Lapko *et al*., 1999; Han *et al*., 2010). To cover our knowledge on phyA, phosphorylation in detail is outside the scope of this work; also, it was reviewed recently (Choi *et al*., 2023). Instead, here we focus on the recent advances in the phosphorylation of plant phyB. We summarize (1) the identification of phosphorylated phyB residues; (2) the functional consequences of these PTMs; (3) our knowledge on phosphatases that can dephosphorylate phyB and (4) studies that identified kinases phosphorylating phyB under different conditions. These recent findings have considerably expanded our knowledge on how phosphorylation of different phyB residues can modify different aspects of light signaling and how these processes can intertwine light signaling with other pathways.

Phosphorylation of phyB

In 2013, two pioneering studies identified different *Arabidopsis thaliana* phyB residues that are phosphorylated and also showed that changing the phospho-status of these amino acids alters phyB function and thus modulates light signaling affecting plant growth and development (Medzihradszky *et al*., 2013; Nito *et al*., 2013).

Medzihradszky *et al*. showed that phyB is phosphorylated *in vivo* on many amino acids. One of these, serine 86 (S86), is located in the NTE domain, and its phosphorylation impairs phyB signaling by accelerating thermal reversion of phyB Pfr (Medzihradszky *et al*., 2013). Thermal reversion deactivates the photoreceptor; thus, phyB Pfr stability has a pronounced role under those conditions when light-induced generation of Pfr is limited, that is under dim light illumination or in the dark. Furthermore, higher temperature destabilizes Pfr, allowing phyB to react as a light-sensitive thermo-sensor, altering development and growth according to the combined effects of ambient light and temperature (Jung *et al*., 2016; Legris *et al*., 2016), and this is how the phosphorylation of S86 affects plant growth under various light and temperature combinations (Viczián *et al*., 2020).

Nito *et al*. identified a motif in the phyB molecule that contains several phosphorylated amino acids, and named it as 'phosphorylation cluster of signaling modulation' (PCSM; Fig. 1a). They examined how phosphorylation of these residues affects signaling and found that phosphorylation of tyrosine 104 (Y104) has the

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most severe phenotype among them. Y104 is phosphorylated to higher levels in light, and its phosphorylation interferes with phyB-PIF3 binding, destabilizes nuclear photobodies and seriously impairs phyB signaling throughout the whole life of plants. The similar role of this residue in other phytochromes is indicated by its conserved position (Nito *et al.*, 2013) although experimental data to confirm this hypothesis are still missing. In this aspect, we note that recently, it has been demonstrated that, similarly to S86, phosphorylation of Y104 also decreases phyB Pfr stability, indicating at least partly overlapping mode of action in regulating light signaling (Viczián *et al.*, 2020).

Additional *in vivo* analyses revealed that phyB is phosphorylated not exclusively within but also outside PCSM, throughout the molecule (Fig. 1a,b). Interestingly, most of the phosphorylated amino acids are located in the NTE domain of the molecule close to PCSM. The phosphorylation status of many of these residues depends on light and temperature conditions and affects thermal reversion. This allows plants to fine-tune signaling by dynamic phosphorylation of these residues, thus modifying phyB activity by setting the amount of available active Pfr (Viczián *et al.*, 2020).

Early studies identified enzymes modifying phytochrome phosphorylation status

The HKRD motif of phytochromes was identified based on sequence similarities to bacterial histidine kinases (Schneider-Poetsch *et al.*, 1991) and, together with the kinase activity of ancient cyanobacterial and algal phytochrome homologs (Yeh *et al.*, 1997; Yeh & Lagarias, 1998; Duanmu *et al.*, 2014), made it tempting to speculate that plant phytochromes act as kinases. This idea was supported by a large set of studies demonstrating that phyA can phosphorylate different proteins and suggesting that phyA also autophosphorylates (Wong *et al.*, 1986; Wong & Lagarias, 1989; Ahmad *et al.*, 1998; Yeh & Lagarias, 1998; Fankhauser *et al.*, 1999; Colón-Carmona *et al.*, 2000; Shen *et al.*, 2009; Shin *et al.*, 2016; Hoang *et al.*, 2021). We note that in all of these *in vitro* studies, oat phyA was examined, and interestingly, Burgie *et al.* (2023) were unable to detect kinase activity of Arabidopsis phyA *in vitro*. In contrast to the numerous studies performed on phyA, investigating kinase activity of phyB has drawn much less attention. A notable exception is the work of Shin *et al.* (2016) demonstrating that Arabidopsis phyB and its close homolog phyD phosphorylate Histone H1 and PIF3 in an *in vitro* experimental system. Interestingly, other *in vitro* approaches failed to detect kinase activity of phyB (Li *et al.*, 2022) and we also point out that kinase activity of phyB has not yet been observed *in planta*. Taken together, we conclude that validation of kinase activity of phyB and determination of its biological significance requires additional experimental evidence.

Direct binding of phytochromes to protein phosphatases was first demonstrated nearly two decades ago. It was shown that oat phyA and Arabidopsis phyB interact with the Ser/Thr-specific protein phosphatases, FyPPs (flower-specific, phytochrome-associated protein phosphatases). FyPPs can dephosphorylate oat phyA *in vitro* (Kim *et al.*, 2002); however, such activity on phyB still awaits for confirmation.

More phyB-specific data are available around two other phosphatases. (1) PAPP5, a type 5 serine/threonine protein phosphatase, dephosphorylates oat *phyA in vitro*. PAPP5 binds to phyB *in vitro* and also *in vivo* and with higher affinity to the Pfr form of phyB; moreover, PAPP5-phyB co-localization can be observed in nuclear PBs under light illumination when phyB signaling is active. Conclusively, these results indicate that PAPP5 may reduce the phosphorylation level of phyB, leading to improved signaling (Ryu *et al.*, 2005). (2) Similar to PAPP5, PAPP2C (phytochrome-associated protein phosphatase type 2C) also interacts with phyB, both *in vitro* and *in vivo* in the nucleus, and additionally, it dephosphorylates phyB *in vitro* (Phee *et al.*, 2008). In plant phenotyping assays, both PAPP5 and PAPP2c appeared as positive regulators of phytochrome signaling: Most probably, they could dephosphorylate the photoreceptor and thereby releasing the inhibition of signaling caused by phosphorylation. Notwithstanding these observations, site specificity or *in planta* dephosphorylation of phyB by these phosphatases have not been confirmed (Table 1).

Salt stress and light signaling are linked by the kinase activity of FERONIA

Whereas phosphatases that interact with phyB have been identified years ago, kinases that bind to phyB and phosphorylate-designated residues were only identified very recently (Table 1; Liu *et al.*, 2023; Zhao *et al.*, 2023).

FERONIA (FER) directly binds to and phosphorylates phyB. It was shown that FER preferably interacts with and phosphorylates the cytosolic Pr form of phyB. An *in vivo* experimental approach identified serines at positions 106 and 227 (S106, S227) of phyB as the main targets of FER. The kinase action of FER promotes phyB-containing photobody dissociation and a decrease in the amount of nuclear phyB after transferring seedlings from light to dark. This could be explained by the faster thermal reversion of phosphorylated phyB what was measured *in vitro*. When the FER pathway is active and FER phosphorylates phyB, phyB signaling is impaired, leading to a hyposensitive phenotypic response (longer hypocotyl), especially under low-intensity R irradiation (Fig. 2a; Liu *et al.*, 2023). The straightforward connection between phyB signaling and salt stress is really intriguing. FERONIA is involved in maintaining cell wall integrity, plant immunity and hormonal balance under salt stress (Stegmann *et al.*, 2017; Feng *et al.*, 2018; Dünser *et al.*, 2019; Zhao *et al.*, 2021). Furthermore, it was observed that *phyB* mutation can rescue some (but not all) phenotypic defects of the *fer* mutant, including salt sensitivity. This can be explained by the altered kinase activity of FER under salt stress, when the FER-dependent phosphorylation of phyB is impaired, leading to elevated amounts of phyB and increased PB stability. This results in increased phyB signaling associated with phenotypic traits such as reduced plant growth, a characteristic phenomenon observed under salt stress (Liu *et al.*, 2023). The above data firmly establish FER as negative regulator of phyB yet the discovery was surprising in the sense that whereas phyB functions mainly require its nuclear localization, FER is a plasma membrane-localized, receptor-like kinase and phosphorylates

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Table 1 Proteins involved in the phosphorylation/dephosphorylation of phytochrome B (phyB).

Identifier	Gene name		Action on phyB	Action on phyA	Reference
AT3G19980 (AF275664)	AtFYPP3	Flower-specific, phytochrome-associated protein phosphatase	Interacts with phyB *in vitro*	Interacts with phyA *in vitro*	Kim *et al*. (2002)
AF305635	FYPP	Flower-specific, phytochrome-associated protein phosphatase		Interacts with and dephosphorylates phyA *in vitro*	
AT2G42810	PAPP5	Phytochrome-associated protein phosphatase 5	Interacts with phyB *in vitro* and *in vivo*, colocalizes with phyB in PBs	Interacts with Arabidopsis phyA *in vitro* and dephosphorylates oat phyA *in vitro*	Ryu *et al*. (2005)
AT1G22280	PAPP2C	Phytochrome-associated protein phosphatase type 2C	Interacts with and dephosphorylate Arabidopsis phyB *in vitro*, colocalizes with phyB in PBs	Interacts with and dephosphorylates oat phyA *in vitro*	Phee *et al*. (2008)
AT3G51550	FER	FERONIA	Interacts with phyB *in vivo*, phosphorylates S106 and S227, regulates Pfr stability and PB formation	None confirmed	Liu *et al*. (2023)
AT2G17290 AT5G23580	CPK6 CPK12	Calcium-dependent protein kinase 6 Calcium-dependent protein kinase 12	Interacts with phyB *in vivo*, phosphorylates S80 and S106 and promotes phyB nuclear import	None confirmed	Zhao *et al*. (2023)

Those proteins are listed in the table that are confirmed to be involved or might be involved in modifying the phospho-state of phyB. The corresponding text sections contain more details.

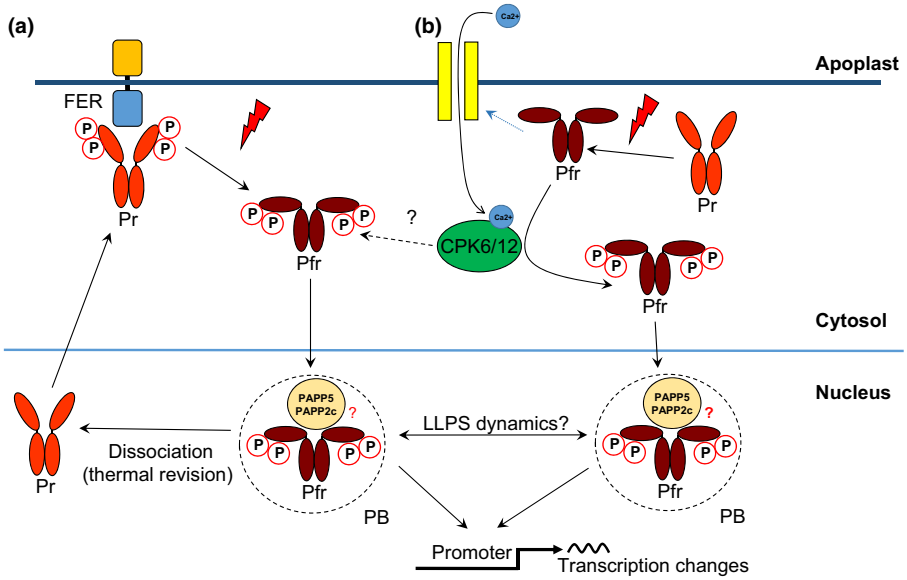

Fig. 2 Simplified model depicting how FERONIA (FER) and CPK6/12 kinases modify phytochrome B (phyB) signaling in Arabidopsis. (a) FER, a membrane-bound kinase, phosphorylates phyB Pr at S106 and S227. Upon R light illumination, Pfr is formed that is translocated to the nucleus. It associates with other proteins in PBs and mediates light-dependent gene expression. Phosphorylated S106 and S227 are necessary for proper photobody dissociation and phyB turnover. (b) Cytoplasmic Ca^{2+} concentration rises upon red light irradiation by a yet unknown phyB action (dotted blue arrow) and activates CPK6 and CPK12, and then, they interact with phyB that they phosphorylate at S80 and S106. These steps are necessary for phyB nuclear import at the early stages of de-etiolation. It is not known whether FER and CPK6/12 compete for phosphorylation of overlapping targets (dashed arrow with question mark). Red flash symbols indicate R light that induces Pfr formation. PAPP2c and PAPP5 phosphatases are co-localized with phyB in the NBs, but the molecular details and functional consequences of these interactions are not yet known. This is indicated by the red question marks. CPK, calcium-dependent protein kinase; LLPS, liquid-liquid phase-separation; P, phosphate group; PB, photobody; Pfr, Pfr form of phyB; Pr, Pr form of phyB.

cytoplasmic, inactive phyB Pr. These facts add new layers of complexity of phyB action. First, there are no confirmed data indicating that a fraction of phyB Pr is membrane bound and even speculations were not made how this pool of phyB could affect signaling. The first data indicating membrane-bound phytochromes were obtained more than 4 decades ago (Marme, 1977), yet these data should be interpreted with care as technical limitations prevented isolation of phyB separately from phyA. To answer the intriguing questions about the function and size of membrane-bound phyB pool and whether beside FER are

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there other factors (notably membrane-bound kinases) that can tether phyB Pr to the membrane require additional experiments.

Phosphorylation of phyB connects lights and Ca^{2+} signaling

Ca^{2+} is an intracellular secondary messenger, and changes in cytosolic Ca^{2+} concentration were observed in response to environmental signals, among them R light, suggesting the involvement of phytochrome-mediated pathways (Shacklock *et al.*, 1992; Neuhaus *et al.*, 1993; Bowler *et al.*, 1994). Thirty years after these observations, a recent study revealed the molecular mechanism that links Ca^{2+} and phyB signaling to each other (Zhao *et al.*, 2023). Red light increases cytosolic Ca^{2+} concentration in a phyB-dependent manner, and elevated Ca^{2+} is required for the light-induced nuclear import of phyB. Calcium-dependent protein kinases (CPKs) bind to Ca^{2+} and phosphorylate their targets. Two of these, CPK6 and CPK12 (CPK6/12), are associated with phyB and specifically phosphorylate S80 and S106 of phyB. The phosphorylation of these residues is an essential prerequisite of the nuclear import of phyB, and the early de-etiolation responses that happen immediately after the transfer of young seedlings from dark to light. The proper phosphorylation of S80 and S106 is necessary only for the nuclear translocation of phyB and does not alter its activity (Fig. 2b). Interestingly, after prolonged exposure to red light, plants lacking functional CPK6/12 show normal nuclear translocation of phyB and hypocotyl elongation, indicating that the action of CPK6/12 is temporary and important only after dark-to-light transition (Zhao *et al.*, 2023). During later phases of development, after prolonged irradiation, the phospho-state of S80 and S106 may be regulated by other kinases, or may not modify phyB import directly.

General conclusions, remaining questions and perspectives

Phosphorylation is a temporary PTM, providing an excellent regulatory tool of diverse responses by the concerted action of kinases and phosphatases. Similarly to phyA, phosphorylated residues of phyB were identified mostly around the NTE domain. To date, only these phospho-residues have been examined, whereas the possible role of phosphorylated amino acids in other domains is unclear (Fig. 1). It is well-established that phosphorylation of the NTE impairs phyA signaling by reducing phyA protein stability (Ryu *et al.*, 2005; Trupkin *et al.*, 2007). NTE phosphorylation also impairs phyB signaling, mainly by fastening thermal reversion and thereby decreasing the amount of available active Pfr. It seems a general way of signal attenuation, since the phosphorylation of different NTE residues has similar effects. Although Pfr stability is an intrinsic property of the molecule, there are proteins that modify it (Sweere *et al.*, 2001; Enderle *et al.*, 2017). Kinases phosphorylating NTE can also modify Pfr stability, and it is expected that besides FER, others will be identified, because FER does not modify other serines, and the modification of Y104 requires another type of kinase. The regulation of the thermal reversion of phyB differs from that of phyA signaling by phosphorylated NTE. The thermal reversion of phyA is a less important factor than that of phyB, because phyA Pfr is a less stable protein than phyB Pfr and it is primarily degradation that limits the available amount of phyA Pfr rather than its reversion to Pr (Rausenberger *et al.*, 2011).

Phosphorylation of S80 and S106 by CPK6/12 in the NTE modulates signaling differently: It induces the nuclear import of phyB and promotes phyB signaling. To date, this is the only phytochrome phosphorylation event that has a positive effect on light signaling. Furthermore, similar mechanisms and responses are observed at homologous positions of phyD and phyE, close homologs of phyB, but not in the case of the phylogenetically more distant phyA or phyC (Zhao *et al.*, 2023). Phosphorylation of phyD and phyE results in decreasing Pfr stability leading to similar physiological responses as of phyB, indicating the universality of this process among these phytochromes even throughout different species (Medzihradszky *et al.*, 2013; Nito *et al.*, 2013; Viczián *et al.*, 2020; Zhao *et al.*, 2023). Together with this, the identified kinases that phosphorylate phyB are also conserved throughout different species (Boudsocq & Sheen, 2013; Franck *et al.*, 2018), suggesting that similar kinases might phosphorylate amino acids in similar positions of phyB-type phytochromes of different species. Conclusively, we might speculate that kinase-phytochrome connections form stable regulatory modules even in evolutionary timeframes. It will be exciting to find out how plants can separate R light-induced Ca^{2+} elevation from Ca^{2+} elevation caused by other environmental stress impacts and prevent active phyB pathways from keeping cytoplasmic Ca^{2+} constantly high under prolonged irradiation. Maybe this unknown mechanism is connected to the regulation of phyB import that requires other, CPK6/12-independent mechanisms (Pfeiffer *et al.*, 2012).

The nuclear translocation of phytochrome is an essential step of light signaling, and nuclear phyB actions are in the forefront of many research efforts. We note, however, that phyB-phosphorylating kinases identified so far are located in the cytoplasm (CPK6/12) or the cell membrane (FER). We cannot exclude that other, yet unidentified, kinases modify the phospho-state of phyB in the nucleus. This idea is supported by the observations on PAPP2C and PAPP5 phosphatases that are active in the nucleus (Ryu *et al.*, 2005; Phee *et al.*, 2008; Fig. 2) and that phyB interacts with a kinase in the nucleus that phosphorylates PIFs resulting in modified light signaling (Ma *et al.*, 2023). Thus on the one hand, nuclear phyB phosphorylation is an important subject to study and, specifically, phosphorylation of nuclear phyB is expected to be detected. On the other hand, the available data point to the importance of phyB PTMs happening outside the nucleus and demonstrate that these can modify light signaling considerably.

A recent study lends extra importance to examining the phosphorylation of phyB NTE. Chen *et al.* demonstrated that phyB PBs that are essential for phyB light and thermo-signaling are liquid–liquid phase-separated (LLPS) droplets. They also showed that the heavily phosphorylated NTE is an intrinsically disordered region that is necessary for the formation of these LLPSs (Chen *et al.*, 2022). Maybe the generally elevated phospho-state of the NTE is necessary to induce LLPS formation or maintain LLPS stability, and we predict that precise phosphorylation 'mapping' of

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phyB NTE will expand our knowledge of photobody dynamics and the corresponding phyB signaling.

Intrinsically disordered regions, like the NTE, are frequently targets of phosphorylation and maybe this PTM sets the disorder-to-order or vice versa transitions (Iakoucheva *et al.*, 2004; Kulkarni *et al.*, 2017; Seok, 2021; Newcombe *et al.*, 2022). Based on these observations, recent phyB structural studies (Burgie *et al.*, 2014; Li *et al.*, 2022) might help to reveal the consequences of phosphorylation on how molecular rearrangements can modify signaling, for example by altering Pfr stability, by providing binding surfaces of interacting proteins, etc. Notwithstanding these new data, it is too early to draw conclusions from them, mainly because directly obtained structure data from phosphorylated phyB has not yet been published. It is technically challenging, because only a small portion of the phyB pool is phosphorylated referred to certain residues *in vivo* (Viczián *et al.*, 2020). Thus, maybe the structure of phospho-mimic mutant phyB versions could reflect the structural changes affecting signaling induced by phosphorylation.

It could be rewarding to revisit the interpretations of experimental data obtained by expressing the N-terminal (PSM) and truncated N-terminal fragments of PHYB in transgenic plants (Matsushita *et al.*, 2003; Oka *et al.*, 2004; Palágyi *et al.*, 2010; Viczián *et al.*, 2020) on the basis of phosphorylation. We do not know whether those amino acids are phosphorylated in these PHYB versions that are phosphorylated in the full length molecule. The phospho-profile of these molecules supplemented by structural studies could help to reveal intimate relations of the different phospho-sites of phyB and the possible *in vivo* function of the HKRD module.

Available data indicate that different phyB residues are phosphorylated by different kinases, and these kinases are really specific in many respects. On the one hand, they are site-specific: for example CPK6/12 phosphorylate S80, but not S84 or S86 in spite of them being in close proximity (Zhao *et al.*, 2023). Similarly, when S86 is mutated, the neighboring intact S84 cannot overtake its functions (Medzihradszky *et al.*, 2013). On this basis, we assume that further kinases will be identified that target specific residues of phyB. On the other hand, the activities of these kinases are specifically regulated by different pathways. Thus, they are joint components of these pathways and phyB signaling. For example, FER can connect salt and light signaling, or CPK6/12 can intertwine Ca^{2+} and light signaling. Additionally, FER signaling modifies Ca^{2+} levels in connection with a wide range of responses (Ngo *et al.*, 2014; Shih *et al.*, 2014; Li *et al.*, 2016; Feng *et al.*, 2018). We cannot exclude that FER and CPKs have combined kinase activity on phyB under special circumstances and their co-action tunes the intensity of the response. As phyB signaling is involved in different responses provoked by environmental stresses (e.g. heat, cold, drought and pathogen attack; Kim *et al.*, 2021), we expect that further kinases will be identified that connect these diverse environmental and light signaling pathways via phosphorylating specific amino acids of phyB.

The effect of kinases on light signaling may be regulated through their activity, (expression level, possible PTM, etc.), or even by the phospho-state of other residues around their target amino acid. Kinases can even compete for their specific site, for example S106 is a shared target of FER and CPK6/12. It is a really interesting finding that S106 and S227 phosphorylation by FER impairs phyB signaling, whereas S80 and S106 phosphorylation by CPK6/12 promotes phyB responses. Thus, the phosphorylation of S106 can negatively or positively modulate phyB-driven responses by tuning the thermal reversion or the nuclear import, respectively. These data imply that the physiological effect of a phosphorylated amino acid could be set by the phospho-state of neighboring residues and thus specific phospho-patterns are formed according to the environmental parameters. These patterns may add quantitative aspects to the signaling by tuning the intensity of the response according to the proportion of phosphorylated phyB molecules referred to certain residues. First, corresponding results indicate that it will be worth to produce and evaluate more quantitative data of this kind (Viczián *et al.*, 2020).

Conclusively, phyB may act as a 'phospho-signaling hub' that governs signaling pathways according to the phospho-state of different residues set by different kinases and phosphatases regulated according to the environmental conditions. Investigating the corresponding regulatory pathways will be a challenge of the future and it will expand our knowledge on the fine-tuning of complex light signaling responses via the phosphorylation of the phyB photoreceptor.

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Competing interests

None declared.

Author contributions

AV conceived the project and wrote the manuscript. FN revised the manuscript. All authors read, contributed to and approved the final manuscript.

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