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SUMOylation of different targets fine-tunes phytochrome signaling

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Summary

Plants monitor their surrounding ambient light environment by specialized photoreceptor proteins. Among them, phytochromes monitor red and far-red light. These molecules perceive photons, undergo a conformational change and regulate diverse light signaling pathways resulting in the mediation of key developmental and growth responses throughout the whole life of plants. Post-translational modifications of the photoreceptors and their signaling partners may modify their function. For example, the regulatory role of phosphorylation has been investigated for decades by using different methodological approaches. In the past few years a set of studies revealed that ubiquitin-like short protein molecules, called SUMOs (Small Ubiquitin-like MOdifier) are attached reversibly to different members of phytochrome signaling pathways, including phytochrome B, dominant receptor of red light signaling. Furthermore, SUMO attachment modifies the action of the target proteins leading to altered light signaling and photomorphogenesis. This review summarizes recent results regarding SUMOylation of various target proteins, the regulation of their SUMOylation level, and the physiological consequences of SUMO attachment. Potential future research directions are also discussed.

1 Introduction

Light plays a key role in the life of plants affecting almost all major developmental steps, resulting in a completely different phenotype grown in the dark (skotomorphogenesis) or under light (photomorphogenesis). Plants developed light-sensitive photoreceptor molecules to perceive light. Each photoreceptor monitors a certain wavelength range allowing the plants to sense ultraviolet B radiation by

UVR8 (UV-B resistance 8), blue light by cryptochrome, phototropin and zeitlupe-like receptors and the red/far-red range by phytochromes (PHY) (Galvão & Fankhauser, 2015). The widely used model plant *Arabidopsis thaliana* possesses five phytochromes (phyA-E) which share high sequence and structural homology but fulfil different physiological roles. Whereas phyA is the main receptor under far-red or very weak illumination of any wavelength, phyB is the dominant receptor of red light signaling (Sharrock & Clack, 2002; Legris *et al.*, 2019). Phytochromes are synthesized in their inactive Pr conformer that can be converted to the biologically active Pfr form upon red light (λ_{max}=~660 nm) illumination. Far-red light (λ_{max}=~730 nm) can convert Pfr back to Pr resulting in switching off signaling (Rockwell *et al.*, 2006). The thermodynamically unstable Pfr can transform to Pr spontaneously, allowing phytochromes to integrate light and temperature signals (Klose *et al.*, 2020). Phytochromes localize to the cytosol in the dark and translocate into the nucleus upon light irradiation. This is a key moment in the initiation of phytochrome-mediated photomorphogenesis. Phytochromes reorganize and inactivate the COP1/SPA (CONSTITUTIVE PHOTOMORPHOGENIC 1 / SUPPRESSOR OF PHYA-105) protein complex that acts as a central repressor of light signaling by targeting positive regulators of photomorphogenesis for degradation, preventing light-induced development in the dark (Zhu *et al.*, 2008; Sheerin *et al.*, 2015; Hoecker, 2017; Han *et al.*, 2020). In the nucleus, phytochrome Pfr interacts with further signaling partners, for example with a set of bHLH (basic helix-loop-helix) transcription factors known as PIF (PHYTOCHROME INTERACTING FACTOR) proteins (Leivar & Monte, 2014). Members of the PIF family promote skotomorphogenesis, negatively regulate photomorphogenesis, furthermore act as a "signaling hub" contributing to the crosstalk between light, thermal, circadian, defense and hormonal signaling pathways (Leivar *et al.*, 2008; Shin *et al.*, 2009; Leivar & Quail, 2011).

Post-translational modifications (PTMs) occur during or after translation, resulting in the covalent modification of proteins. In most of the cases, PTMs are reversible and induce alterations in the function of the target protein allowing to control fast regulatory responses. Attachment of Small Ubiquitin-like Modifier (SUMO) proteins is a PTM that occurs among eukaryotes. SUMO and ubiquitin proteins show no sequence homology but have high structural similarity. Ubiquitination typically leads to the proteasomal degradation of the target protein (Vierstra, 2009), whereas SUMO conjugation may result in changes in stability, altered functionality, intracellular localization, interaction to different partners or nucleic acids, etc. of the SUMOylated target protein (Augustine & Vierstra, 2018). Arabidopsis expresses four SUMO isoforms (SUMO1–3 and SUMO5) and our knowledge about the differences in their function is rudimentary (van den Burg *et al.*, 2010; Hammoudi *et al.*, 2016). SUMO attachment to the target lysine residue requires the

consecutive action of a set of enzymes. After the activation of SUMO by the SUMO activation enzyme (SAE) SUMO is transferred to the SUMO conjugation enzyme 1 (SCE1). The E3 SUMO ligases (HIGHPLOIDY2 (HPY2) and SAP & MIZ1 DOMAIN CONTAINING LIGASE 1 (SIZ1) in Arabidopsis) attach SUMO to a lysine residue located typically in a SUMOylation consensus motif, ψ-K-X-E/D (ψ: hydrophobic amino acid, K: acceptor lysine, X: any residue, D: aspartic acid, E: glutamic acid) (Novatchkova *et al.*, 2004; Miura & Hasegawa, 2010; Park *et al.*, 2011; Vierstra, 2012; Castaño-Miquel *et al.*, 2013; Tomanov *et al.*, 2014).

Whereas SUMOylation is performed by only a few enzymes, the removal of SUMO from the target protein (de-SUMOylation) is done by at least seven identified SUMO proteases. This fact, together with the genome analyses that predict the existence of other SUMO proteases, suggests that the control of de-SUMOylation has key importance in the regulation of target SUMOylation level (Mukhopadhyay & Dasso, 2007; Hermkes *et al.*, 2011; Novatchkova *et al.*, 2012; Yates *et al.*, 2016). The data collected indicate that SUMOylation is essential for normal growth and development, and the general SUMOylation of the plant proteome is increased under developmental changes and various stress responses (Kurepa *et al.*, 2003; Murtas *et al.*, 2003; Miura *et al.*, 2005; Lee *et al.*, 2007; Saracco *et al.*, 2007; Jin *et al.*, 2008; Conti *et al.*, 2008; Miller *et al.*, 2010, 2013; Castro *et al.*, 2012; Elrouby, 2015; Bailey *et al.*, 2016; Cai *et al.*, 2017; Castaño-Miquel *et al.*, 2017; Rytz *et al.*, 2018; Verma *et al.*, 2018).

Large-scale analyses helped us identify SUMOylated proteins and assess the general dynamics of SUMOylation (Budhiraja *et al.*, 2009; Miller *et al.*, 2010; Elrouby & Coupland, 2010; Park *et al.*, 2011; López-Torrejón *et al.*, 2013), but functional analyses of this PTM requires detailed examination of the physiological consequences of SUMOylation on individual targets. In the past few years, this latter research approach was extended over phytochromes and their signaling partners. The present work summarizes our current knowledge on how phytochrome signaling is fine-tuned by SUMOylation of different components of the signaling cascades. We collect the physiological consequences of SUMOylation focusing on its modifying effect on photomorphogenic development.

2 PhyB SUMOylation Attenuates Light Signaling

The first report that turned attention to the role of SUMOylation in light signaling demonstrated that phyB was SUMOylated at the acceptor site lysine 996 (K996) and the phyB(K996R) mutant showed negligible amount of SUMOylation compared with the wild-type phyB (Sadanandom *et al.*, 2015). Replacing the SUMO attachment site lysine with arginine (K-R mutation) is a general method to create non-SUMOylatable

molecules, and its importance is highlighted because “SUMO-mimic”, unlike phosphor-mimic mutants cannot be generated. The SUMOylation level of the phyB pool increases under red or white light irradiation, when a high proportion of the molecules are in the active Pfr conformation. This observation indicates that SUMOylation has a role in phyB signaling, and this notion is further supported by the fact that phyB localized in the nucleus has elevated SUMOylation level. PhyB(K996R) and phyB have the same photoconversion properties and intracellular localization, but red-light-grown seedlings that express the phyB(K996R) have increased inhibition of hypocotyl elongation and larger cotyledons compared with the expressors of the wild-type counterpart. Furthermore, phyB(K996R) binds to PIF5 with higher affinity and accumulates to lower levels under prolonged red irradiation than SUMOylated wild type phyB (Sadanandom *et al.*, 2015). This is an interesting observation, because phyB Pfr binding to PIFs leads to the co-degradation of both proteins, regulating the level of available phyB and contributing to proper photomorphogenic development (Khanna *et al.*, 2004; Ni *et al.*, 2013, 2014). Conclusively, phyB SUMOylation attenuates light signaling, presumably by compromising the binding of phyB to PIF5 and most probably to other PIFs (Figure 1A).

Our knowledge about the dynamics of phyB SUMOylation is rudimentary, but a solid set of data is available on phyB de-SUMOylation. The SUMO protease, OVERLY TOLERANT TO SALT 1 (OTS1) binds to and de-SUMOylates phyB. SUMOylated phyB is accumulated in *ots1ots2* mutant that does not contain functional OTS1 and its closest homologue, OTS2. Furthermore, *ots1ots2* seedlings show hyposensitive photomorphogenic phenotype in red light, compared with wild-type plants (Sadanandom *et al.*, 2015). Beyond this role of OTS 1 and 2 in photomorphogenic development, these SUMO proteases are also necessary for proper plant growth under salt stress (Conti *et al.*, 2008). This observation raises the possibility how the de-SUMOylation activity of OTS1/2 can interconnect light and abiotic stress signaling pathways.

3 phyB signaling is modified by the SUMOylation of phyB Signaling Partners

PIF transcription factors accumulate to high levels and repress photomorphogenesis in darkness. Binding of phyB Pfr to PIFs is one of the earliest molecular event of photomorphogenesis resulting in changes of PIFs’ DNA binding, rapid phosphorylation, ubiquitination and subsequent degradation. It was also observed that the co-degradation of PIFs and phyB regulates phyB levels under prolonged red irradiation (Khanna *et al.*, 2004; Bauer *et al.*, 2004; Shin *et al.*, 2009; Zhang *et al.*, 2013; Ni *et al.*, 2013, 2014).

A recent work demonstrates that PIF3 is SUMOylated at the acceptor lysine 13, thus phosphorylation and ubiquitination are not the only PTMs of PIF3 (Bernula *et al.*, 2021). Whereas phosphorylation happens

after the onset of light illumination and leads to the fast degradation of PIF3, SUMOylation occurs both in the light and dark reaching higher levels in the dark and does not modify the light-induced degradation of the molecule. Interestingly, plants grown under prolonged red light irradiation and expressing the SUMO acceptor site mutant PIF3(K13R) contained less phyB than those that express wild-type PIF3, indicating that PIF3 SUMOylation alters the PIF3-phyB co-degradation. Additionally, PIF3(K13R) bound with higher affinity to the target promoters than SUMOylated PIF3 (Figure 1A). As PIF3 negatively regulates seedling photomorphogenesis, the strong hyposensitive responses of PIF3(K13R)-expressing light-grown seedlings suggest that SUMO attachment reduces the biological activity of PIF3, thus promotes photomorphogenesis (Bernula *et al.*, 2021).

SUMOylation of other PIFs has not been demonstrated so far, but a recent study shows how SUMOylation can modify PIF4 signaling. Originally PIF4 was identified as a repressor of phyB signaling (Huq & Quail, 2002), but forthcoming studies showed that it also mediates responses under blue and UV-B irradiation independent of phyB (Hayes *et al.*, 2014; Ma *et al.*, 2016; Boccaccini *et al.*, 2020) and integrates these light pathways with thermomorphogenesis (Quint *et al.*, 2016; Zhang *et al.*, 2017). Similarly to PIF3, PIF4 interacts with phyB Pfr resulting in rapid PIF4 phosphorylation, ubiquitination and degradation (Lorrain *et al.*, 2008).

PIF4 interacts with SEUSS (SEU), a negative regulator of photomorphogenesis. SEU is a transcriptional activator that regulates gene expression in various developmental processes (Franks *et al.*, 2002; Sridhar *et al.*, 2004, 2006; Pfluger & Zambryski, 2004; Grigorova *et al.*, 2011; Gong *et al.*, 2016; Huai *et al.*, 2018). SEU and PIF4 directly associate with the chromatin and regulate the expression of several hundred genes, including auxin biosynthetic and responsive genes. SEU is required for the PIF4 action on gene regulation and cell growth, and together they regulate light- and temperature-dependent development (Huai *et al.*, 2018). Recently it was demonstrated that SEU is SUMOylated *in planta* and its SUMOylation is increased in light resulting in elevated functionality and stability of SEU. The SEU(4KR) mutant – having all four potential SUMO attachment lysines (K170, K200, K216, K392) substituted by arginines – shows no detectable SUMOylation. SEU(4KR) cannot complement the *seu* mutant phenotype, binds stronger to PIF4 than the wild-type SEU and decreases PIF4 function on transcription mediation. Currently available data suggest that SUMO binding to SEU impairs the SEU-PIF4 interaction and it is essential for proper function of both SEU and PIF4. The amount of SEU protein and its SUMOylation level increase in light when it interacts with phyB Pfr (Zhang *et al.*, 2020). These findings, together with the phyB-induced PIF4 degradation in light indicate that phyB controls SEU

action by the complete rearrangement of the SEU-PIF4 complex. This is modified by the SUMOylation of SEU at several points, regulating PIF4 function indirectly but the details of this process is not known (Figure 1A).

4 SUMOylation modifies phyA signaling

SUMOylation of phyA has not been demonstrated so far, but a recent study described how this PTM can modulate phyA signaling (Qu *et al.*, 2020). It is a characteristic feature of phyA signaling that it is more effective under far-red or very low fluences of any light irradiation when the Pfr levels are low, compared with strong red light when Pfr reaches higher levels. The highly sophisticated regulation of this mechanism was puzzled out by explaining how the instability of the phyA Pfr together with its binding to and releasing from its specialized nuclear transport facilitators FAR-RED ELONGATED HYPOCOTYL 1 (FHY1) and FHY1-LIKE (FHL) are responsible for proper phyA action (Rausenberger *et al.*, 2011). FHY1 and FHL are small homologous plant-specific proteins that are responsible for the nuclear translocation of phyA, with FHY1 having the predominant function. They each contain a phyA-binding domain, nuclear localization and nuclear export signal allowing them to cross the nuclear membrane easily. Their key importance for proper phyA signaling is demonstrated by the fact that the *fhy1fhl* mutant containing no functional FHY1 and FHL resembles the *phyA* mutant under FR irradiation (Zeidler *et al.*, 2004; Hiltbrunner *et al.*, 2005, 2006; Zhou *et al.*, 2005; Rösler *et al.*, 2007; Genoud *et al.*, 2008; Menon *et al.*, 2020). Additional data support that appropriate FHY1/FHL protein levels and their trafficking between the nucleus and cytosol are necessary for proper phyA signaling (Rausenberger *et al.*, 2011).

A recently published report demonstrates that the amount of available FHY1 is regulated by SUMOylation (Qu *et al.*, 2020). SUMOs can be attached to lysines 32 and 103 of FHY1 resulting in higher instability of the protein, but not impairing its binding affinity to phyA Pfr. The non-SUMOylatable FHY1(K32R K103R) protein *in planta* (i) accumulates to higher levels than its wild-type counterpart, (ii) can complement the mutant phenotype and (iii) mediate enhanced photomorphogenesis in far-red, similarly to the FHY1 overexpressors. In the nucleus, FHY1 interacts with the ARABIDOPSIS SUMO PROTEASE 1 (ASP1) that mediates the de-SUMOylation of FHY1 resulting in higher FHY1 levels and enhanced FR signaling. Transcription and protein levels of ASP1 are negatively regulated by far-red light providing a negative feedback to ASP1 action on FHY1 (Qu *et al.*, 2020). In conclusion, FHY1 SUMOylation desensitizes phyA signaling by destabilizing FHY1 leading to its increased degradation (Figure 1B).

5 Question of dark and light: SUMOylation of COP1 Maintains Skotomorphogenic Development

COP1 is an E3 ubiquitin ligase and in cooperation with SPA proteins (SPA1-4) it ubiquitinates positive regulators of photomorphogenesis in the dark. COP1 targets mostly transcription factors, inducing their proteasomal degradation, thus maintaining skotomorphogenesis (Han *et al.*, 2020). In the light, COP1 binds to phytochromes directly and ubiquitinates them (Seo *et al.*, 2004; Jang *et al.*, 2010; Viczián *et al.*, 2012; Sheerin *et al.*, 2015). Light illumination also results in inactivation of COP1 ubiquitin ligase activity by reorganizing the COP1/SPA complex and changing the intracellular distribution of COP1, excluding it – although not entirely – from the nucleus (von Arnim *et al.*, 1997; Stacey *et al.*, 1999; Yu *et al.*, 2013; Pacín *et al.*, 2014).

A recent study demonstrated that COP1 was SUMOylated at lysine 193 by the SIZ1 SUMO ligase and SUMOylated COP1 accumulates in the dark. SUMOylation enhances ubiquitin ligase activity of COP1, but does not alter COP1's protein stability, substrate specificity or intracellular localization (Lin *et al.*, 2016; Mazur *et al.*, 2019). Plants expressing the non-SUMOylated COP1(K193R) mutant protein contain higher levels of photomorphogenesis-promoting factors and show enhanced photomorphogenic development. Interestingly, COP1 ubiquitinates SIZ1 resulting in decreased amounts of SIZ1 (Figure 1C). Thus COP1 reduces its own level of SUMOylation and activity (Lin *et al.*, 2016). It was also observed that by controlling the available amount of SIZ1, COP1 can regulate the general SUMOylation of the proteome under abiotic stress conditions (Kim *et al.*, 2016). An increasing amount of data suggests that COP1 SUMOylation and its action on SIZ1 can connect light signaling with diverse stress responses, hormonal signaling pathways and miRNA biogenesis (Luo *et al.*, 2010; Jeong *et al.*, 2010; Cho *et al.*, 2014; Chico *et al.*, 2014).

6 Discussion and Future Perspectives

The surprisingly diverse physiological consequences of SUMOylation require the examination of each signaling component individually. Proteome-based approaches will result in valuable global and general but rather limited outcomes with regards to the molecular function and physiological significance. Based on the results available so far, SUMOylation modifies phytochrome signaling at three groups of targets: (i) photoreceptors; (ii) transcriptional regulating components and (iii) other signaling partners (Table 1.).

6.1 SUMOylation of photoreceptors.

SUMO attachment to phyB impairs photomorphogenic responses most probably by impairing phyB binding to PIF transcription factors. The SUMO acceptor lysine is located at the C-terminal part of phyB and interestingly, this part of the molecule is involved in the regulation of PIF protein stability (Qiu *et al.*, 2017). Similarities in the SUMO acceptor site among PHYB sequences of different species indicate the universal nature of phyB SUMOylation (Sadanandom *et al.*, 2015) and *in silico* data suggest that other phytochromes can also be SUMO targets (Table 2). It is also tempting to speculate that SUMOylation alters the formation of phytochrome heterodimers, providing an extra fine-tuning mechanism to phytochrome light sensing. Furthermore, a recent report demonstrated that the blue light photoreceptor phototropin 2 is SUMOylated, but its physiological significance is not known (Łabuz *et al.*, 2021), indicating that SUMO modification of plant photoreceptors is not exclusive to phyB but is a general fine-tuning mechanism of light signaling (Table 2).

The major positive regulators of light signaling are the active conformers of light sensing photoreceptors. Interestingly, most of their signaling partners negatively regulate light signaling. Based on the available data, we speculate that SUMOylation of the receptors themselves cannot enhance receptor function by improving their signaling capability but rather attenuate signaling. Thus this PTM has the same effect on light signaling as the action of photomorphogenesis inhibitors, but the details of this process are not known. We hope that further investigations will expand our view on the subject by testing/analyzing the physiological role of the yet only predicted SUMO targets (Table 2).

6.2 SUMOylation of phytochrome-regulated transcription complex components.

Binding of phyB to PIFs is an important step of photomorphogenesis, and whereas PIF3 SUMOylation does not interfere with its phyB-binding affinity, SUMOylation of phyB inhibits binding of PIF5. The available data also suggest that phyB stability is regulated by the SUMOylation state of phyB and PIFs, most probably via the phyB-PIF co-degradation mechanism (Sadanandom *et al.*, 2015; Bernula *et al.*, 2021). It is interesting to note that SUMOylation reduces the activity of both phyB and PIF3, resulting in different outcomes on photomorphogenesis. SUMOylation of phyB impairs, whereas SUMOylation of PIF3 enhances photomorphogenesis (Table 1). This is due to the opposite roles of phyB and PIF3: phyB promotes, whereas PIF3 inhibits photomorphogenic development. Further studies are required to find out whether the

SUMOylation or de-SUMOylation of these proteins are performed by the same enzymes, allowing an interesting possibility of regulating the same pathway at different components.

Our knowledge about the transcriptional activity of PIFs is rather rudimentary. Two interesting observations suggest that SUMOylation regulates this mechanism through different targets: (i) SUMOylation of PIF3 decreases PIF3 binding affinity to target promoters (Bernula *et al.*, 2021) and (ii) SUMOylation of SEU impairs its binding to PIF4, thus interferes with PIF4 function (Zhang *et al.*, 2020). In both cases the outcome is similar: SUMOylation moderates PIFs action on target DNA. This, together with PIFs' decreased stability caused by phyB Pfr binding results in reorganization of phyB-PIF complexes thus mediating the developmental switch from skoto- to photomorphogenesis (Figure 1A). PIFs are also involved in mediating blue/UV responses independently of phytochromes and also mediate physiological responses of plants to hormonal, temperature and stress stimuli. Future studies will reveal how potential SUMOylation of other PIFs and their binding of other SUMOylated proteins (Table 2) contribute to PIF function in these responses.

6.3 SUMOylation of different signaling modifiers.

Phytochrome signaling can be regulated by the SUMOylation of their partners with diverse functions.

For example, SUMOylation of COP1, a ubiquitin ligase increases its ubiquitination activity on positive components of photomorphogenesis, directing them to proteasomal degradation in darkness (Lin *et al.*, 2016). Under light irradiation, however, this action of COP1 must be reduced for proper photomorphogenesis. To achieve this, light-activated phyB and phyA interact directly with COP1 and their action reorganizes the COP1/SPA signaling complex. The COP1 SUMOylation level decreases in light resulting in decreased COP1 activity, and the vast majority of COP1 is excluded from the nucleus (Figure 1C). Unfortunately, the available data are still insufficient to describe precisely those phytochrome-dependent molecular mechanisms that are altered by SUMOylation of COP1. COP1 ubiquitinates many different target proteins with diverse functions, thus regulation of COP1 by SUMOylation may influence different signaling pathways other than photomorphogenesis. Furthermore, COP1 ubiquitinates SIZ1 regulating the available amount of SUMO protease for mediating other developmental, stress etc. pathways. To reveal the fine details of these regulatory mechanisms will be challenging but interesting tasks in the future.

SUMOylation of FHY1 is an example of how SUMOylation can modify phyA signaling without the direct modification of phyA itself. FHY1 is a small protein necessary for the nuclear translocation of phyA. SUMOylation decreases its stability resulting in a lower amount of FHY1 and, in conclusion, impaired phyA

nuclear import of and signaling by phyA (Figure 1B). Several studies reported that FHY1 can mediate signaling by direct binding to transcription factors that are signaling components of phyA-mediated responses, and phyA can also associate with FHY1 and chromatin elements at the same time (Yang *et al.*, 2009; Chen *et al.*, 2012, 2014; Jang *et al.*, 2013). Further investigations are necessary to reveal whether FHY1 SUMOylation can modify FHY1 function in these protein-DNA complexes.

6.4 Future perspectives

An obvious approach to expand our knowledge on the role of SUMOylation in light signaling is the identification of further SUMO target proteins and SUMO attachment site lysines. Web-based applications are available to identify SUMOylatable lysines, and current data shows that SUMOylation might be more prevalent than expected from the available experimental data (Table 2). Furthermore, our knowledge about the occurrence, dynamics and functional consequences of poly-SUMOylation (formation of SUMO chains) at certain targets involved in light signaling is scarce, and this would be an interesting research subject in the future.

A possible avenue for future research direction could be the examination of the interplay of SUMOylation and other PTMs. For example, phyB, COP1, PIF3 and FHY1 are also phosphorylated and their phosphorylation modifies photomorphogenesis (Shen *et al.*, 2009; Chen *et al.*, 2012; Ni *et al.*, 2013, 2017; Lin *et al.*, 2017; Viczián *et al.*, 2020). We have to note, however, that the interplay of phosphorylation and SUMOylation of these proteins is not studied and despite recent advances (Verma *et al.,* 2021) our general knowledge about the coordinated action of these PTMs on the biological activity of a target protein is rather limited. Similarly, we do know how the interplay of SUMOylation and ubiquitination can modify light signaling, although based on reports from other experimental systems we might expect future studies focusing on this subject (Lamoliatte *et al.*, 2017; Rott *et al.*, 2017).

Besides the identification of further SUMO targets among phytochrome signaling partners, a very interesting aspect for future research is the determination of the SUMOylation/de-SUMOylation dynamics of the targets. The number of SUMO proteases is higher than that of the enzymes involved in SUMO activation/conjugation/ligation. Thus regulating the de-SUMOylation of a target protein offers wider possibilities than the regulation of SUMOylation. The SUMO proteases identified in light signaling are also involved in other responses. For example: (i) OTS1/2 de-SUMOylate phyB, thus promote photomorphogenesis and furthermore also coordinate salt tolerance (Conti *et al.*, 2008) and (ii) ASP1 promotes phyA signaling by

de-SUMOylating FHY1 and also promotes flowering through its SUMO protease activity (Kong *et al.*, 2017). It is also tempting to speculate that the regulation of de-SUMOylation by other environmental/developmental pathways may modify light signaling.

SUMO attachment may modify the direct interaction of the target protein with its partners as we see above between phyB and PIF5 or between SEU and PIF4. Noncovalent interactions with SUMOs can be mediated by short consensus sequences called SUMO-interacting motifs (SIMs). The hydrophobic core of SIMs offers a highly specific binding moiety for SUMO (Kerscher, 2007). *In silico* prediction data (Table 2) support the speculation that SIMs may take part in the assembly of the above-mentioned protein complexes, but the presence of SIMs and their role in light signaling components have not been experimentally confirmed and studied.

In the last decades the major phytochrome signaling components were identified, and their connection with each other in the signaling pathways were intensively examined. Now working models help to fit new data into a general picture, but our knowledge about the fine tuning of these pathways is still rudimentary. Post-translational modifications may modify the action of the target protein leading to inaccuracies in the predictions. Our expanding knowledge about the physiological consequences of these PTMs will lead to better understanding of light signaling mechanisms.

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8 Author Contributions

A.V. conceived the project and wrote the manuscript; C.P. performed data analysis; N.F. revised the manuscript. All authors read, contributed to and approved the final manuscript.

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10 Figure legend

Figure 1. Schematic effects of SUMOylation on light signaling components

This Figure summarizes the main initiation steps of photomorphogenic development affected by SUMOylation. **(A)** PhyB-dependent pathways affected by SUMOylation. Cytoplasmic phyB Pr can be transported to the nucleus after light-induced photoconversion to Pfr. Nuclear phyB Pfr maintains phyB signaling. Pfr-Pr conversion in the nucleus leads to phyB export to the cytoplasm. phyB Pfr is preferably SUMOylated and SUMOylated phyB binds to PIF5 with lower affinity (indicated by the smaller PIF5 symbol) and is more stable than its non-SUMOylated counterpart. OTS1/2 can de-SUMOylate phyB, and the SUMO ligase mediating phyB SUMOylation has not been identified. SEU SUMOylation by SIZ1 is promoted by phyB action. SUMOylation is necessary for proper SEU function, and SUMO attachment to SEU impairs SEU binding to PIF4. phyB Pfr rearranges the SEU/PIF4 complex: it binds to SEU and also to PIF4 and directs PIF4 to proteolytic degradation. These molecular processes are necessary for normal photo- and thermomorphogenesis. The SUMOylation level of PIF3 is higher in the dark than in the light. SUMOylated PIF3 binds to the target promoters with lower affinity. phyB Pfr attenuates PIF3 signaling by inducing PIF3 degradation and inhibiting PIF3 binding to promoters. phyB Pfr binds to both SUMOylated and non-SUMOylated PIF3 with similar affinity, but phyB degradation happens more efficiently when PIF3 is not SUMOylated. **(B)** Effect of SUMOylation on far-red signaling. Upon light irradiation phyA Pfr interacts with FHY1, a nuclear transporter in the cytoplasm. After their joint nuclear import FHY1 can be exported back the

cytoplasm and recycled. Nuclear FHY1 is preferably SUMOylated in the light that increases FHY1 instability, reducing its amount. FHY1 is de-SUMOylated by ASP1, which is under phyA control. Nuclear phyA Pfr regulates gene expression necessary for photomorphogenic development. **(C)** COP signaling is modulated by SUMOylation. COP1 ubiquitinates positive components of photomorphogenesis to prevent photomorphogenic development in the dark. SIZ1 SUMOylates COP1 preferably in the dark and this modified COP1 is more effective than non-SUMOylated COP1. COP1 also ubiquitinates SIZ1. Pfr phytochromes (both phyA and phyB) inhibit COP1 action and initiate COP1 export from the nucleus allowing an increase in the amount of photomorphogenesis promoters.

Notes: (i) thicker/thinner arrows indicate more/less pronounced action, respectively (e.g. comparing SUMOylation/de-SUMOylation or the action of the SUMOylated/non-SUMOylated form of the same protein); (ii) After the nuclear import of phyB Pfr, it was not tested to what extent the SUMOylated phyB contributes to interactions with PIF3, SEU and COP1, thus phyB SUMOylation is not indicated at these steps.

Table 1. Experimentally analyzed SUMOylated components of phytochrome signaling pathways.

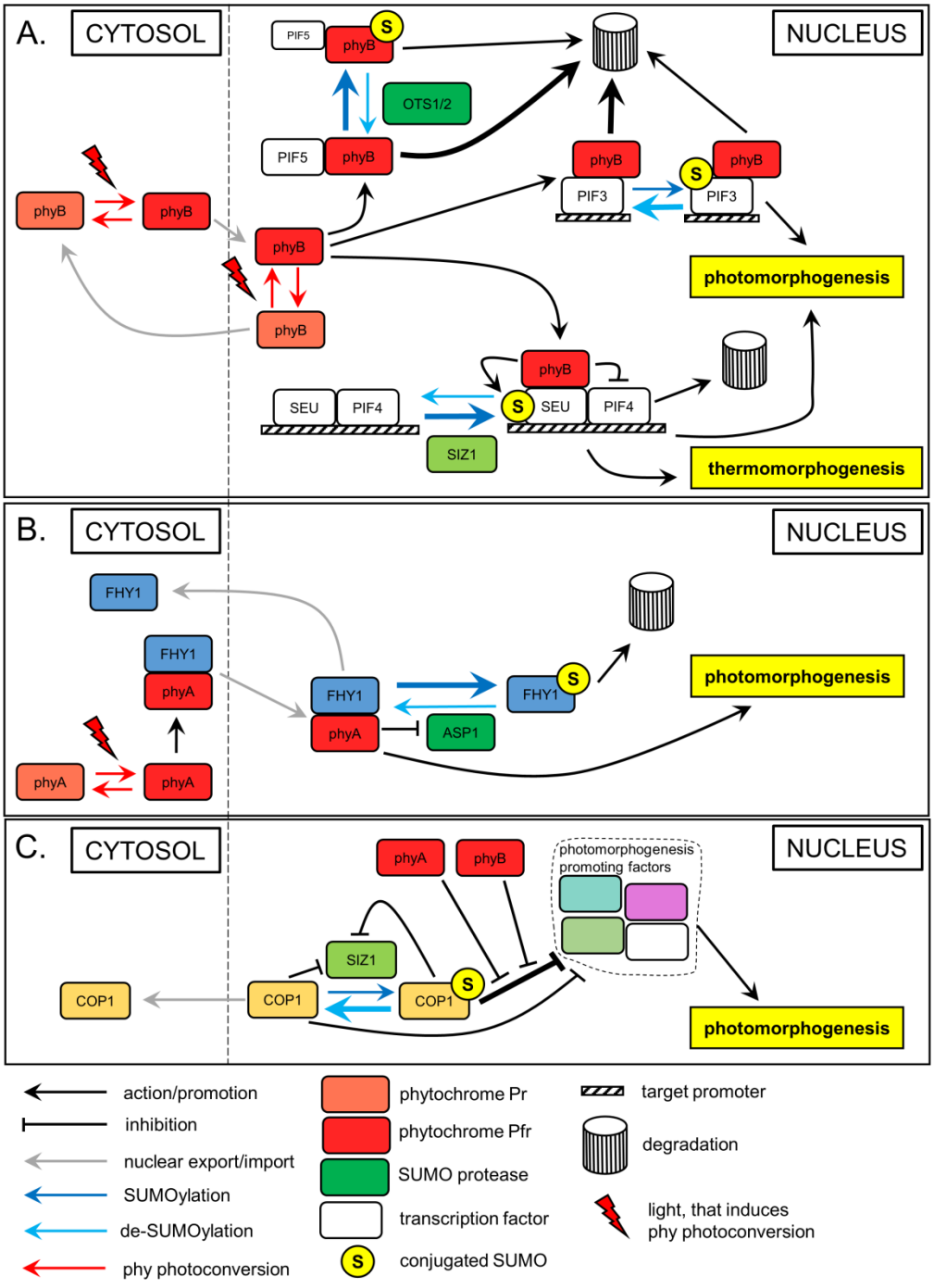
n.d. :not determined

Table 2. Predicted SUMO attachment sites and SIM motifs of selected Arabidopsis proteins involved in light signalling.

SUMO attachment site lysines and amino acid positions of the SUMO interacting motifs (SIM) were identified by the current versions (as of 05.05.2021) of the SUMOplot (https://www.abcepta.com/sumoplot) and GPS-SUMO (http://sumosp.biocuckoo.org/online.php, (Zhao *et al.*, 2014) web-based online tools. The attachments sites identified as low probability or non-consensus are not listed.

Protein	SUMOylated lysine	SUMO ligase / protease	SUMOylation is higher in	SUMOylation's effect on protein action / photomorphogenesis	Reference
PHYB	K996	n.d. / OTS1/2	light	inhibition / inhibition	Sadanandom *et al* ., 2015
PIF3	K13	n.d. / n.d.	dark	inhibition / promotion	Bernula *et al* ., 2021
COP1	K193	SIZ1 / n.d.	dark	promotion / inhibition	Lin *et al* ., 2016
SEU	K170, K200, K216, K392	SIZ1 / n.d.	light	promotion / inhibition	Zhang *et al* ., 2020
FHY1	K32, K103	n.d. / ASP1	light	inhibition / inhibition	Qu *et al* ., 2020

protein	AGI code	SUMOplot	GPS-SUMO	
		SUMO attachment site (high probability)	consensus SUMO attachment site	SIM position
photoreceptors				
PHYA	AT1G09570	K444, K536, K608, K789	K444, K536, K608. K789	178-182, 674-678
PHYB	AT2G18790	K475, K562, K845, K940, K996	K475, K996	213-217, 332-336, 589-593, 1144-1148
PHYC	AT5G35840	K435, K522, K735, K775, K895	K435, K735, K775	81-85, 174-178, 421-425, 585-589, 1103-1107
PHYD	AT4G16250	K566, K725, K849, K944	K725	216-220, 347-351, 593-597, 1148-1152
PHYE	AT4G18130	K306, K429, K517, K883	K429	706-710
CRY1	AT4G08920	no	no	39-43, 361-365, 610-614
CRY2	AT1G04400	K2, K422, K516, K544	K2, K544	358-362, 471-475
CRY3	AT5G24850	K166, K382, K539	K309	no
PHOT1	AT3G45780	K89, K125, K284, K344, K595, K761, K790, K910	K125, K84, K761, K790	736-740
PHOT2	AT5G58140	K79, K191, K220, K297, K704, K711, K897	K79, K220, K297, K704, K711, K897	593-597, 884-888, 903-907
UVR8	AT5G63860	no	no	17-21, 69-73
ZTL	AT5G57360	K381	no	59-63, 150-154, 323-327, 428-432, 611-615
FKF1	AT1G68050	K393	no	159-163, 440-444
LKP2	AT2G18915	K159	no	59-63, 324-328, 429-433
PIFs				
PIF1	AT2G20180	no	no	no
PIF2=PIL1	AT2G46970	K27, K140	K27	86-90, 134-138
PIF3	AT1G09530	K13	K13	29-33
PIF4	AT2G43010	no	no	27-31
PIF5	AT3G59060	K132	K132	29-33
PIF6	AT3G62090	K140	no	no
PIF7	AT5G61270	no	no	no
PIF8	AT4G00050	no	no	no
FHY1/FHL				
FHY1	AT2G37678	K32, K103	K32, K103	no
FHL	AT5G02200	no	no	22-26
COP1/SPAs				
COP1	AT2G32950	K14, K193	K14, K193, K273, K653	444-448, 499-503, 670-674
SPA1	AT2G46340	K102, K191, K287, K338, K525, K884	K287, K525,	397-401, 789-793
SPA2	AT4G11110	K31, K308	K31, K308	72-76, 172-176, 604-608, 1030-1034
SPA3	AT3G15354	K266, K522, K526	K522	101-105, 830-834
SPA4	AT1G53090	K472, K476, K518	K472	89-93, 788-792
SEU homologs / paralogs				
SEU	AT1G43850	K170, K200, K216, K392, K565	K170, K200, K216, K392	no
SLK1	AT4G25520	K106, K583	K106	275-279
SLK2	AT5G62090	no	no	no
SLK3	AT4G25515	K78	K78	247-251
LUG	AT4G32551	K22, K228, K300, K786	K22, K300, K786	318-322, 915-919, 964-968
LUH	AT2G32700	K145, K203	K203	752-756



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