Light-regulated Development in *Arabidopsis*

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ABSTRACT

Many external and internal factors affect multiple developmental processes in plants. Light is one of the essential factors that regulates various aspects of plant growth and development throughout their life cycle. Through combination of genetic and biochemical assays, this doctoral work elucidated the role of B-box protein 21 (BBX21) in the crosstalk between light and abscisic acid (ABA) network, and identified two novel CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) regulators which are responsible for modulation of COP1 abundance and/or activity.

In the model plant *Arabidopsis thaliana*, light is sensed by various photoreceptors and promotes photomorphogenesis. A subset of BBX proteins, including BBX4, BBX20, BBX21and BBX22, play positive roles in the light signaling pathway. Plant hormones are also involved in photomorphogenesis, and several regulators functioning in the light signaling participate in the hormone pathways as well. To increase the knowledge of BBX proteins in the crosstalk between light and hormones, in **Paper I** the role of BBX21 in response to light and ABA was investigated. *Arabidopsis* mutants for *BBX21* gene are hyposensitive to light, in **Paper I** they were found to be hypersensitive to ABA. BBX21 physically interacted and formed heterodimers with ELONGATED HYPOCOTYL 5 (HY5) or ABA INSENSITIVE 5 (ABI5), thereby interfering with HY5 or ABI5 binding to the *ABI5* promoter to activate *ABI5* or *ABI5*-regulated genes expression.

COP1 is a key repressor of plant photomorphogenesis. In the dark, COP1 targets many downstream substrates for ubiquitination and promotes their degradation via the 26S proteasome system to repress photomorphogenesis. To explore candidate genes responsible for regulation of COP1, in **Paper II** and **III** we performed a genetic screen for suppressors of *cop1*. Two novel COP1 regulators were identified and characterized, namely COP1 SUPPRESSOR 1 (CSU1) and CSU2. Either *csu1* or *csu2* alone completely suppressed constitutive photomorphogenic phenotype of *cop1-6* in the dark.

CSU1, which is a Ring-finger E3 ubiquitin ligase, co-localized with COP1 in nuclear speckles, and negatively regulated the abundance of COP1 in the dark. CSU1 was able to ubiquitinate COP1 *in vitro* and was found to be responsible for the COP1 ubiquitination *in vivo*. Thus, CSU1 acts as an E3 ubiquitin ligase to maintain the COP1 homeostasis in the dark-grown seedlings **(Paper II)**.

CSU2 encoding a coiled-coil protein, physically interacted and co-localized with COP1 in the nuclear speckles through their coiled-coil domains. CSU2-COP1 association resulted in the repression of the E3 ubiquitin ligase activity of COP1 towards HY5 **(Paper III)**.

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List of papers discussed

This thesis is based on the following papers, which will be referred to by their roman numerals in the text:

Paper I

Xu D[1]., Li J[1]., Gangappa N.S., Hettiarachchi C., Lin F., Andersson X.M., Jiang Y., Deng X.W., and Holm M. (2014). Convergence of light and ABA signaling on the *ABI5* promoter. PloS Genetics 10 (2): e1004197.

Paper II

Xu D., Lin F., Jiang Y., Huang X., Li J., Ling J., Hettiarachchi C., Tellgren-Roth C., Holm M., and Deng X.W. (2014). The RING-finger E3 ubiquitin ligase COP1 SUPPRESSOR 1 negatively regulates COP1 abundance in maintaining COP1 homeostasis in dark-grown *Arabidopsis* seedlings. Plant Cell 26 (5): 1981-1991.

Paper III

Xu D., Lin F., Jiang Y., Ling J., Hettiarachchi C., Tellgren-Roth C., Holm M., and Deng X.W. *Arabidopsis* COP1 SUPPRESSOR 2 represses COP1 activity through their coiled-coil domain association to optimize COP1-mediated development. Manuscript.

[1] Both authors contributed equally to this work

Abbreviations

ABA	Abscisic Acid
BR	Brassinosteroid
CK	Cytokinin
GA	Gibberellin
JA	Jasmonic acid
EMS	Ethyl methanesulfonate
ABI5	ABA Insensitive 5
BBX	B-Box proteins
CRY	Cryptochrome
CO	Constans
COP1	Constitutively Photomorphogenic 1
CIP	COP1 Interacting Protein
CSU	COP1 Suppressor
DET1	De-etiolated 1
ELF3	Early Flowering 3
GI	Gigantea
HY5	Elongated Hypocotyl 5
HYH	HY5 Homolog
HFR1	Long Hypocotyl in Far-red light 1
LRB	Light-Response-Bric-a-Brack/ Tramtrack/Broad
LAF1	Long After Far-red light 1
phy	phytochrome
PIF	Phytochrome Interacting Factor
Phot	Phototropin
RUP	Repressor of UV-B Photomorphogenesis
SPA	Suppressor of Phytochrome A-105
SOM	Somnus
UVR8	UV Resistance Locus

CONTENTS

1. INTRODUCTION

1.1 Role of Light throughout the Life Cycle of Plants

Sunlight is an electromagnetic wave, which is a major energy source for plants. Plants perceive the sunlight and produce energy for themselves through photosynthesis. In order to sustain life, other living organisms such as animals, humans, directly or indirectly get most of the energy from the plants. Besides being a energy source, light is one of most important factors that affect all facets of plant development, including seed germination, photomorphogenesis, leaf development, shade avoidance and flowering (Kendrick and Kronenberg, 1994; Wang and Deng, 2003; Jiao et al., 2007). In nature, most of plants begin their life cycle as seeds and sufficient amount of sunlight are needed for seeds to develop into healthy strong plants. Plants have the ability to sense the quality, intensity, wavelength, direction and duration of the ambient light in order to optimize their growth and development (Sullivan and Deng, 2003).

1.2 Photomorphogenesis and Skotomorphogenesis

As illustrated in Figure 1, the development of wild-type *Arabidopsis thaliana* seedling follows two contrasting patterns, skotomorphogenesis (or etiolation) in darkness and photomorphogenesis (or de-etiolation) in the light (Sullivan and Deng, 2003). Dark-grown seedlings develop long hypocotyls, apical hooks, and closed cotyledons with nonphotosynthetic etioplasts. This developmental process is known as skotomorphogenesis or etiolation (Figure 1, left). In the light, photomorphogenic development is initiated and light-grown seedlings have short hypocotyls and expanded green cotyledons with chloroplasts and chlorophyll, a developmental patten known as photomorphogenesis (Figure 1, right). In the natural environment, soil-buried seeds are under dark or very low density of light conditions and undergo skotomorphogenesis to emerge through soil to reach light, which in turn, switch to photomorphogenic development (Frankhauser and Chory, 1997).

1.3 Photoreceptors

The *Arabidopsis* seedlings perceive light through four classes of wavelength specific photoreceptors. (1) red and far-red light absorbing phytochromes (600-750 nm) (Quail et al., 1995; Chory et al., 1996); (2) blue light absorbing

cryptochromes (400-500 nm) (Ahmad and Cashmore, 1996; Lin, 2000); (3) blue and UV-A light absorbing phototropins (315-500 nm) (Briggs et al., 2001; Briggs and Christie, 2002); (4) UV-B light absorbing UVR8 (280-315 nm) (Rizzini et al., 2011) (Figure 2). These photoreceptors perceive, interpret, and transduce light signals and might directly bind to numerous promoters of downstream genes to regulate the expression of hundreds of genes, and eventually leading to produce the appropriate physiological and developmental responses (Tepperman et al., 2001, 2004; Chen et al., 2014).

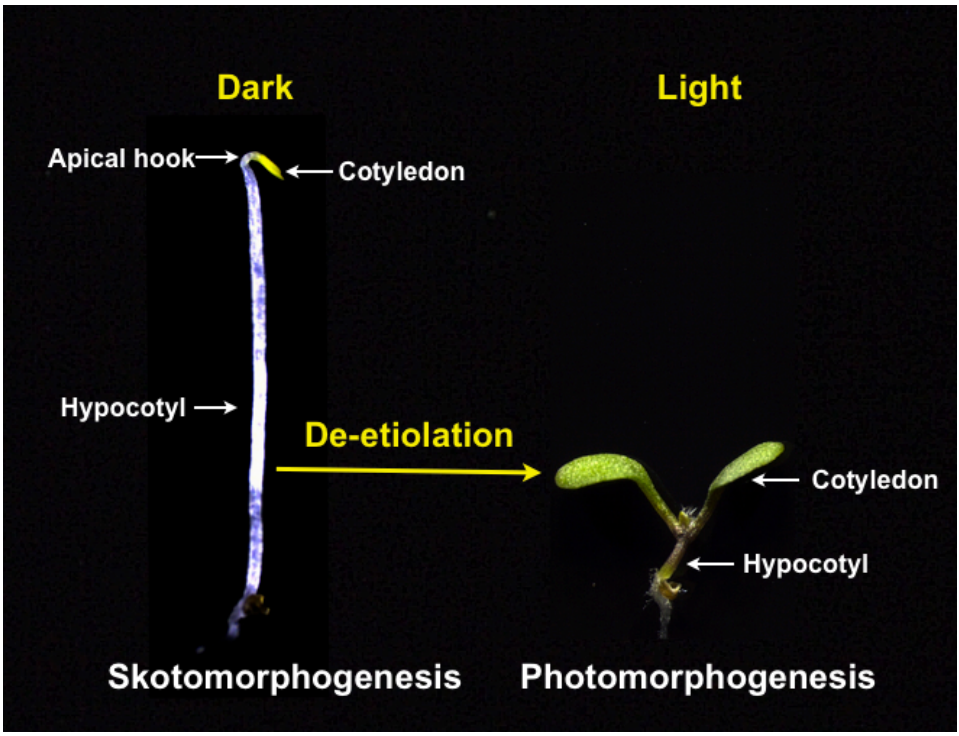

Figure 1. Morphology of *Arabidopisis* seedling grown in the dark and in light.
Wild-type *Arabidopsis* seedling grown in the dark and light undergoes skotomorphogenic (left) and photomorphogenic (right) development, respectively.

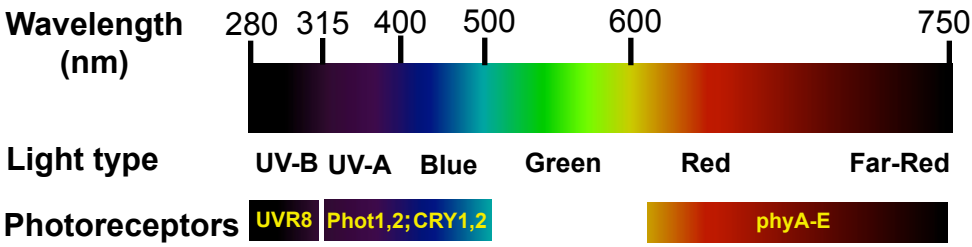

Figure 2. Photoreceptor-mediated light perception in *Arabidopsis*.
A large part of the solar spectrum is perceived by various wavelength specific photoreceptors in *Arabidopsis*. PhyA-E absorb red and far-red light (600-750 nm); CRY1 and CRY2 absorb blue light (400-500 nm); PHOT1 and PHOT2 absorb blue and UV-A light (315-500 nm); UVR8 absorbs UV-B (280-315 nm). Adapted from (Tilbrook et al., 2013) with minor modification.

1.3.1 Phytochromes

In *Arabidopsis,* there are five phytochromes, namely phytochrome (phy) A to E. Phytochromes play critical roles in seed germination, seedling de-etiolation, flowering, gravitropic orientation, circadian clock, stomatal development and shade avoidance (Franklin and Quail, 2010; Li et al., 2011). Phytochromes have two different inter-convertible chemical structures. The Pr form of phy absorbs red light (660 nm) which is biologically inactive and biologically active Pfr form absorbs far-red light (730 nm). phyA is labile in response to light, and phyB-E are light stable phytochromes. phyA and phyB are the most prominent phytochromes and they sense far-red and red light, respectively. In dark-grown seedlings, phyA and phyB localize in the cytoplasm. Upon light exposure, phyA and phyB import into the nucleus and form photobodies (Sharrock and Quail, 1989; Clack et al.,1994; Hirschfeld et al., 1998; Sharrock and Clack, 2002; Li et al., 2011). Tyr 276 His substitution of phyB (phyB^{Y276H}) is a constitutively active phyB version and complements *phyB* mutants. phyB^{Y276H} transgenic seedling display constitutive photomorphogenic phenotype in the dark (Su and Lagarias, 2007).

PHYTOCHROME INTERACTING FACTORs (PIFs) including PIF1 to PIF8, act directly downstream of phytochromes and interact with phytochromes (Castillon et al., 2007). In the dark, biologically active PIFs are enriched and modulate their target genes expression to repress photomorphogenic development (Leivar et al., 2008). Upon light exposure, PIFs interact with Pfr form of phytochromes, are phosphorylated and degraded via the 26S proteasome system, thereby releasing the suppression of photomorphogenesis (Al-Sady et al., 2006; Shen et al., 2007). Phosphorylation of PIF3 initiates the recruitment of

the Light-Response-Bric-a-Brack/Tramtrack/Broad (LRB) Cullin3-type E3 ubiquitin ligases to PIF3-phyB complex, resulting in promoting concurrent polyubiqutination and degradation of both PIF3 and phyB by LRB3 *in vivo* (Ni et al., 2014). Consistently, *pif1/pif3/pif4/pif5* quadruple mutant (*pifq*) seedlings develop constitutive photomorphogenic phenotype in the dark (Leivar et al., 2008).

1.3.2 Cryptochromes

Cryptochromes are blue light receptors which contains two domains, the N-terminal PHR domain (Photolyases Related), and the C-terminal CCT domain (Cryptochrome C-terminal Extension) (Lin and Shalitin, 2003). In *Arabidopsis*, there are two cryptochromes, CRY1 and CRY2, which mediate de-etiolation, stomatal opening, root development, circadian clock, and flowering (Li and Yang, 2007). In the dark, both CRY1 and CRY2 are unphosphorylated, inactive, stable and enriched in the nucleus. Blue light, especially high-fluence blue light, induces phosphorylation of CRY1 and CRY2, thereby leading to translocation of CRY1 into the cytoplasm from the nucleus and degradation of the CRY2, repectively (Guo et al., 1999; Shalitin et al., 2002; Sang et al., 2005). *Arabidopsis* transgenic plants overexpressing $CRY1^{G380R}$, the C-terminal domain of either CRY1 (CCT1) or CRY2 (CCT2) fused to β-glucuronidase (GUS) show constitutive photomorphogenic phenotype in the dark, respectively (Gu et al., 2012; Yang et al., 2000; Wang et al., 2001), and CCT1, CRY1, CCT2 and CRY2 are capable of interacting with COP1 (Yang et al., 2001; Wang et al., 2001). CRY1 is required for the regulation of COP1 nucleocytoplasmic partitioning in response to blue light (Osterlund and Deng, 1998).

1.3.3 Phototropins

Phototropins (Phot1, Phot2) perceive blue and UV-A light (320-500 nm) and contain two LOV (Light, Oxygen, Voltage) domains, and a serine-threonine kinase domain at the C-terminus. LOV domains bind Flovin Mononucleotide (FMN) and possess photochemical activity (Briggs et al., 2001). Phot1 and Phot2 sense the light signal through their LOV domains and mediate various light-controlled development such as phototropism, leaf flattening, leaf positioning, inhibition of hypocotyl growth, chloroplast movement, and stomatal opening (Briggs and Christie, 2002). In the dark, Phot1 and Phot2 are localized in plasma membrane. After blue light irradiation, Phot1 and Pho2 translocate from the plasma membrane to the cytoplasm and the Golgi apparatus, respectively (Sakamoto and Briggs, 2002; Kong et al., 2006). Blue light and

darkness induces auto-phosphorylation or de-phosphorylation of Ser-851 in Phot1 kinase domain, respectively (Inoue et al., 2008). Phot1 is ubiquitnated by the CUL3-Ring E3 ubiquitin ligase, $CRL3^{NPH3}$ in the blue light (Roberts et al., 2011).

1.3.4 UV-B resistance 8 (UVR8)

UVR8 perceives UV-B (280-315 nm) which is an intrinsic part of sunlight (Rizzini et al., 2011). UVR8 protein forms homodimers in the absence of UV-B. Upon UV-B light illumination, the inactive UVR8 homodimers sense the signal through its tryptophan residues (Trp-285), thereby immediately monomerizing into active UVR8 monomer. Monomer UVR8 interacts with COP1 and thus activates transcription of *HY5* to activate UV-B induced genes expression. REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 (RUP1) and RUP2 disrupt the UVR8-COP1 association, allowing reforming the UVR8 homodimers for UV-B perception and thus play a negative feedback role in regulating UVR8 (Gruber et al., 2010; Rizzini et al., 2011; Tilbrook et al., 2013).

1.4 Integration of Light and Hormone Signaling

Plants have evolved complex methods of sensing, integrating external and internal signals. It is not surprising that a number of regulators of seedling photomorphogenesis also participate in plant hormone such as gibberellin (GA), auxin, cytokinin (CK), abscisic acid (ABA), brassinosteroid (BR), and ethylene signaling pathways. Increasing studies uncover how light and hormonal pathways interact at the molecular level, and a range of regulators of light signaling including PIF1, PIF3, PIF4, PIF5, HY5 and a subset of B-box (BBX) proteins, have been identified to play crucial roles in various hormone network as well (Lau and Deng, 2010).

PIFs play critical role in the light signaling and act as repressors of photomorphogenesis. PIF1 reduces the GA responsiveness and levels, and increases ABA levels by positively modulating *DOF AFFECTING GERMINATION1(DAG1)*, *SOMNUS* (*SOM*) and two *DELLA* genes (*GAI* and *RGA*) in the dark, thereby repressing seed germination (Oh et al., 2007; Kim et al., 2008; Gabriele et al., 2010). The highly accumulated GA induces the degradation of GA-signaling repressors, DELLAs, via the 26S proteasome pathway in darkness. However, in the light, decreasing of GA level allows the accumulation of DELLAs, which in turn, sequesters the PIF3 and PIF4 from their target genes, thereby promoting photomorphogenesis (de Lucas et al.,

2008; Feng et al., 2008). PIF4 and PIF5 negatively regulate auxin-mediated phototropism in response to blue light by directly binding to the promoters of *INDOLE-3-ACETIC ACID INDUCIBLE 19* (*IAA19*) and *IAA29* to activate their expression (Sun et al., 2013). In BR signaling, BRASSINOSTEROID INSENSITIVE 2 (BIN2), which is a BR signaling kinase, directly interacts with and phosphorylates PIF4 to promote its destabilisation (Bernardo-García et al., 2014). Thus, PIF1-PIF5 act as integrators in the crosstalk between light and various hormone networks.

HY5 acts as a key crosstalk juncture between light and various hormone transduction networks. GA and CK also control HY5 at the post-transcriptional level in a COP1-dependent manner, and HY5 promotes photomorphogenesis at least in part by modulating auxin, GA and ABA signaling (Lau and Deng, 2010). ABI5 is a positive regulator in the ABA signaling and *ABI5* overexpressors are insensitive to light, suggesting it also functions as a positive regulator of light signaling. HY5 directly binds to the promoter of *ABI5* to activate its transcription. Thus, the HY5-ABI5 regulon represents an integration point for the coordination of seedling development in response to light and ABA (Chen et al., 2008).

A set of BBX proteins act as integrators in the transduction networks of light- and hormone signaling as well (Table 1). BBX16 negatively regulates light-inhibition of hypocotyl elongation under low Red:Far-red (R:FR) conditions, and reduces the Auxin levels by promoting expression of a suppressor of auxin biosynthesis gene, *SUPERROOT 2* (*SUR2*), in high R:FR (Wang et al., 2013; Zhang et al., 2014). BBX18/DDB1a functions as negative regulator of blue light-mediated hypocotyl elongation and is also involved in gibberellin homeostasis. In response to blue light, BBX18 up-regulates metabolic genes *GA3β-hydroxygenase 1* (*GA3ox1*) and *GA20-oxidase1* (*GA20ox1*) expression, and down-regulates catabolic genes *GA2-oxidase1* (*GA2ox1*) and *GA2-oxidase 8* (*GA2ox8*), lead to increasing bioactive GA levels, which in turn, promoting hypocotyl growth (Wang et al., 2011a). BBX20/BZS1 plays a positive role and functions overlapping or redundantly with BBX21 in light signaling, and acts as a negative regulator in BR signaling. BR represses the expression of *BBX20* to promote hypocotyl length growth, whereas light promotes BBX20 protein accumulation to repress the hypocotyl elongation (Fan et al., 2012).

Table 1. BBX proteins mediate various light-regulated development and hormone signaling.

Name	AGI code	Processes involved	Interacting partners	Literature source
BBX1/CO	At5G15840	Flowering; Stomatal opening	COP1, SPA1, HOS1	Laubinger et al.,2006; Jang et al., 2008; Liu et al., 2008
BBX2/COL1	At5G15850	Circadian clock	ND	Ledger et al., 2001
BBX4/COL3	At2G24790	Photomorphogenesis,light signaling; Flowering;Shoot branching; Lateral root development	COP1	Datta et al., 2006
BBX6/COL5	At5G57660	Flowering	ND	Hassidim et al., 2009
BBX7/COL9	At3G07650	Flowering	ND	Cheng et al., 2005
BBX16/COL7	At1G73870	Shoot branching; Auxin signaling	ND	Wang et al., 2013; Zhang et al., 2014
BBX18/ DDB1a	At2G21320	Photomorphogenesis, light signaling; GA signaling	ND	Kumagai et al., 2008; Wang et al., 2011a
BBX19/ DDB1b	At4G38960	Photomorphogenesis, light signaling	ND	Kumagai et al., 2008
BBX20/ DDB2/BSZ1	At4G39070	Photomorphogenesis, light signaling; BR signaling	COP1	Fan et al., 2012
BBX21/STH2	At1G75540	Photomorphogenesis, light signaling; Shade avoidance; ABA signaling	HY5, BBX32	Crocco et al., 2010 Datta et al., 2007 Holtan et al., 2011
BBX22/STH3	At1G78600	Photomorphogenesis, light signaling	HY5, HYH, COP1	Datta et al., 2008
BBX23/ DDB4/ MIDA10	At4G10240	Skotomorphogenesis	ND	Sentandreu et al.,2011
BBX24/STO	At1G06040	Photomorphogenesis, light signaling	HY5,HYH, COP1	Holm et al., 2001; Gangappa et al., 2014
BBX25/STH/ STH1	At2G31380	Photomorphogenesis, light signaling	HY5,HYH, COP1	Holm et al., 2001; Gangappa et al., 2014
BBX32	At3G21150	Photomorphogenesis, light signaling	HY5, BBX21	Holtan et al., 2011

Adapted from (Gangappa and Botto, 2014) with minor modification. ND represents Non-Determined.

1.5 Repressors of Photomorphogenesis

Forward genetic screens for phenotypes affecting light-regulated seedling morphogenesis of *Arabidopsis*, have identified a group of proteins referred to as *CONSTITUTIVE PHOTOMORPHOGENIC/DE-ETIOLATED/FUSCA* (*COP/DET/FUS*). The recessive *cop/det/fus* mutants display constitutive photomorphogenic phenotype in darkness, indicating that they act as repressors of photomorphogenesis (Wei and Deng, 1996). Analysis of genome expression profiles reveal that *cop/det/fus* mutants grown in darkness have the expression profiles which closely resemble those of in light-grown WT seedlings (Ma et al., 2002, 2003). Biochemical characterization of the proteins encoded by these loci define three biochemical complexes, seven of the loci are involved in the biogenesis of the COP9 signalsome, a nuclear protein complex that activates cullin-containing multisubunit ubiquitin ligases (Deng et al 2000; Cope and Deshaies 2003; Wei and Deng, 2003); COP10, an E2 ubiquitin conjugating enzyme variant, forms a 350 kD complex with DAMAGED DNA-BINDING PROTEIN1(DDB1), and DEETIOLATED1 (DET1) that is able to stimulate the ubiquitin conjugating activity of E2 enzymes (Yanagawa et al., 2004); the COP1-SUPPRESSOR OF PHYTOCHROME A-105 (SPA) complex containing two COP1 and two SPA subunits is required for degradation of HY5 in the dark and acts as an E3 ubiquitin ligase in this pathway (Osterlund et al., 2000; Saijo et al., 2003). The COP/DET/FUS play a crucial role in the primary transition between the skotomorphogenic and photomorphogenic developmental pathway, acting to suppress photomorphogenesis in darkness. Of these, the E3 ubiquitin ligase, COP1, is extensively characterized, acting as a master regulator of many facets of plant developmental processes.

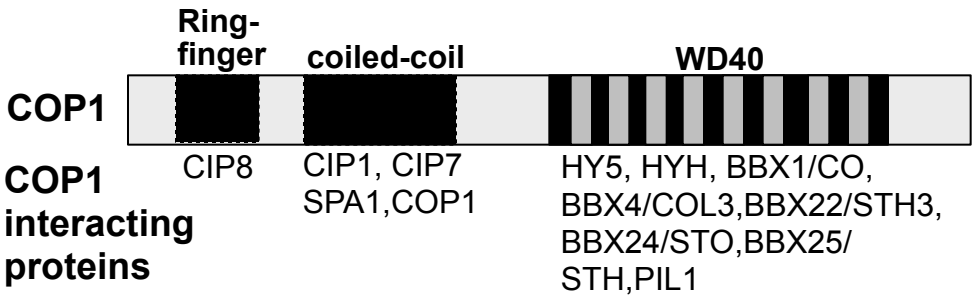

Figure 3. Structural organization and interactions of COP1.
COP1 contains three protein–protein interaction domains: an N-terminal Ring-finger region, a coiled-coil domain and a WD40 domain at its C-terminus. Adapted from (Holm and Deng, 1999) with minor modification.

1.6 COP1 Interacting Proteins

COP1 is a 76 kDa Ring-finger E3 ligase that contains three protein–protein interaction domains: an N-terminal Ring-finger region, a coiled-coil domain and a WD40 domain at its C-terminus (Deng et al., 1992) (Figure 3). During the past two decades, extensive biochemical and genetic studies have identified a number of proteins interact with COP1. So far, the ubiquitin E3 ligase, COP1 Interacting Proteins 8 (CIP8) specially interact with Ring-finger of COP1 (Torii et al.,1999). The Ring-finger of E3 ubiquitin ligase has been shown to interact with E2 ubiquitin-conjugating enzymes which transfer active ubiqtins to E3 ligase (Lorick et al., 1999). Consistently, Cys 52 Ser and Cys 52 Ser substitutions in Ring-finger motif of COP1 abolishes its E3 ubiquitin ligase activity (Seo et al., 2003). While COP1 coiled-coil domain associate with CIP1(Matsui et al., 1995), CIP7 (Yamamoto et al., 1998), as well as SPA, and COP1 itself to form corn stable complexes (Zhu et al., 2008). Thus, coiled-coil domain of COP1 is involved in its dimerisation and formation of COP1-SPA complexes (Torii et al., 1998; Zhu et al., 2008). Notably, most of COP1 interacting proteins specifically interact with WD40 domain of COP1. HY5 (Osterlund et al., 2000), HYH (Holm et al., 2001), BBX1/CO (Jang et al., 2008; Liu et al., 2008), and BBX22/STH3 (Datta et al., 2008), PIL1 (Luo et al., 2014) are ubiquitinated by COP1 for degradation, while BBX24/STO, BBX25/STH (Holm et al., 2002) protein stability are regulated in a COP1-dependent manner, suggesting that WD40 domain of COP1 most likely is responsible for recruiting target proteins for ubiquitination and degradation (Holm and Deng, 1999; Holm et al., 2001, 2002).

1.7 Role of COP1 in Regulating Plant Development

COP1 is characterized and cloned more than 20 years ago (Deng et al., 1991, 1992). COP1 functions as a master regulator in light-mediated plant development, ranging from seedling stage to adult stage. Null *cop1* alleles are lethal at the seedling stage, however, weak alleles display a constitutive photomorphogenic phenotype in darkness and further studies confirm that COP1 is a key repressor of photomorphogenesis (Deng et al., 1991, 1992; McNellis et al., 1994a). Besides the constitutive photomorphogenesis phenotype in darkness, mutations in *COP1* result in pleotropic effects throughout the plant life cycle, including high anthocyanin accumulation, short hypocotyls, short primary root length, dwarf adult plant and early flowering in the light (McNellis et al., 1994a; Nakagawa and Komeda, 2004; Sassi et al., 2012). Extensive biochemical and genetic assays demonstrate that COP1 functions as an E3 ubiquitin ligase and targets a range of photomorphogenesis-promoting factors for ubiquitination and

degradation, thereby repressing the photomorphogenesis in darkness. In the dark, COP1 is enriched in the nucleus and ubiquitinates a set of positive regulators of photomorphogenesis, such as HY5, HYH, LAF1, HFR1, BBX20/ BZS1, BBX22/STH3 and PIL1 for proteasome-mediated degradation (Osterlund et al., 2000; Holm et al., 2002; Saijo et al., 2003; Seo et al., 2003; Jang et al., 2005, 2010; Datta et al., 2008; Fan et al., 2012; Luo et al., 2014). Upon light illumination, COP1 translocates to the cytoplasm and is inactivated. Thus, COP1 target proteins accumulate and promote photomorphogenic development. COP1 is responsible for the ubquitination and degradation of phyB-phyE as well (Jang et al., 2010). Degradation of phyA is dependent on COP1 in the presence of sucrose (Seo et al., 2004; Debrieux et al., 2013). In the *cop1* mutants, more CRY2 protein level accumulates, implying that COP1 negatively regulates CRY2 at post-translational level, though it is not clear whether COP1 directly targets CRY2 for ubiquitination (Shalitin et al., 2002).

Besides repression of photomorphogenesis in darkness, COP1 also plays a critical role in modulating the flowering time, circadian clock, BR and JA signaling (Lau and Deng, 2012). Mutations in *CONSTANS (CO)* suppress early flowering phenotype of *cop1* under short-days (8 h L/16h D). COP1 targets CO for ubiquitination and promotes its degradation during the night to repress flowering (Jang et al., 2008; Liu et al., 2008). In the dark, the highly accumulated EARLY FLOWERING 3 (ELF3) acts as a substrate adaptor and promotes COP1 to interact with circadian clock-associated protein GIGANTEA (GI) *in vivo* and targets it for degradation (Yu et al., 2008). The integrator of light and BR signaling, GATA2, is also ubiquitinated and proteasomally degraded in a COP1-dependent manner (Luo et al., 2010). In addition, the jasmonate (JA)-related transcription factor, MYC2 is also negatively regulated by COP1 in darkness (Chico et al., 2014). Therefore, COP1 participates in multiple plant developmental processes via its E3 ubiquitin ligase activity, and negatively regulates abundance of its downstream substrates (Figure 4 and Table 2).

It has been proposed that light inactivates COP1 by triggering COP1 export from the nucleus to the cytoplasm. Weak *cop1* mutants display more anthocyanin accumulation, short hypocotyls, short roots, dwarf adult phenotype in the light (Deng et al., 1991, 1992; McNellis et al., 1994a; Sassi et al., 2012), indicating that COP1 also plays an essential role in regulating these plant developmental processes. However, the exact molecular role of COP1 in the light remains poorly understood.

In *Arabidopisis,* two SPA and two COP1 proteins form stable core complexes in the dark (Zhu et al., 2008). The SPA family consists of four members, SPA1 to SPA4, that each contain an N-terminal kinase-like domain, followed by a coiled-coil domain and a WD40 domain at the C-terminal. Quadruple *spa* mutants display striking constitutive photomorphogenic phenotype similar to strong *cop1* mutants in the dark (Saijo et al., 2003; Laubinger et al., 2004). SPA1 enhances the COP1 E3 ubiquitin ligase activity on LAF1 when concentration of COP1 is low. Substrates of COP1 including HY5, HFR1 and CO accumulate in the *spa* mutants (Saijo et al., 2003; Yang et al., 2005; Laubinger et al., 2006; Zhu et al., 2008). Since SPA proteins are able to directly interact with HY5, HFR1 and CO through their WD40 domains and SPA1 has litter effect on COP1 self-ubiquitination (Seo et al., 2003; Saijo et al., 2003; Yang et al., 2005; Laubinger et al., 2006), it is possible that SPA proteins enhance COP1 activity through substrates recruitment (Lau and Deng 2012). COP1–SPA complexes bind to DDB1 with their WDxR motifs and act as substrate receptors in the formation of multimeric CUL4–DDB1$^{COP1\text{-}SPA}$ ligases (Chen et al., 2010). CUL4 together with CDD complex form a multimeric CUL4–CDD ligase (Bernhardt et al., 2006; Lee et al., 2008).

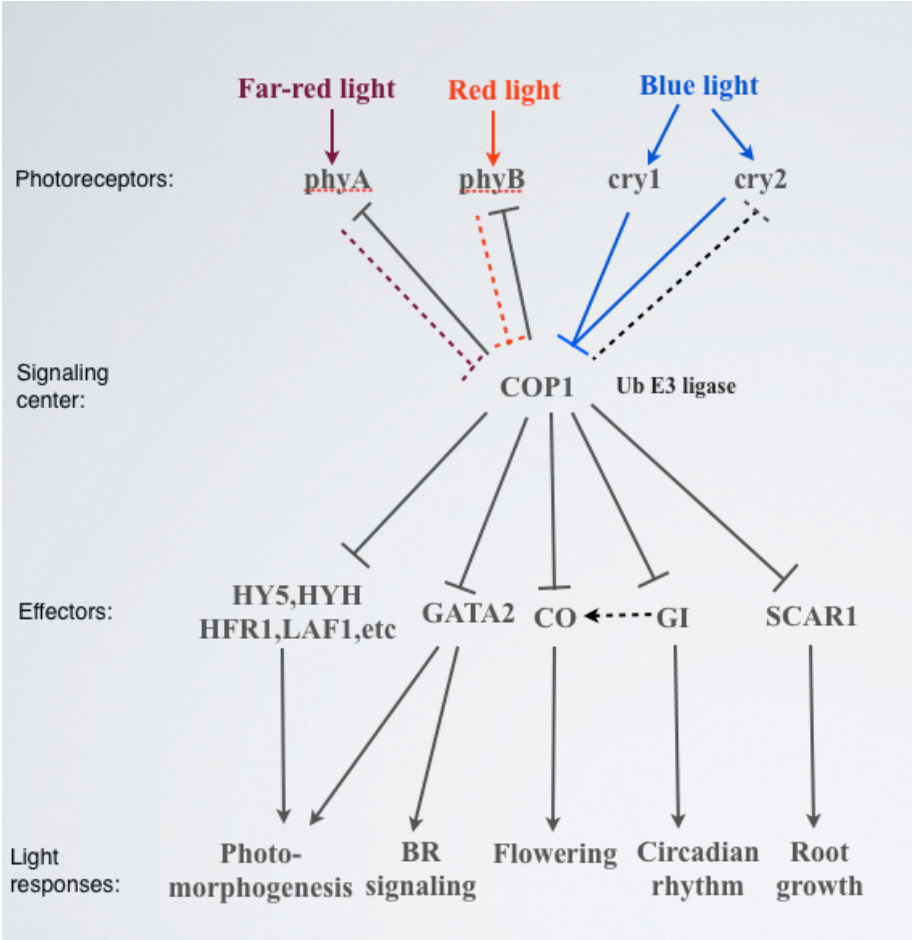

Figure 4. COP1 represents a signaling node that integrates signals from various photoreceptors and also controls many downstream light-regulated responses.
Phytochromes (phyA and phyB) and cryptochromes (cry1 and cry2) are the major photoreceptors that perceive a wide spectrum of visible light. After perceiving light signal, the activated photoreceptors act to suppress COP1. COP1 is a repressor in light signal transduction and functions as an E3 ubiquitin ligase that ubiquitinates multiple light-response effectors for degradation. Thus, photoreceptor-mediated suppression of COP1 allows accumulation of the effectors, resulting in the specific light responses. Adapted from (Lau and Deng 2012) with minor modification.

Table 2. Targets of the COP1 E3 ubiquitin ligase.

Substrate	AGI code	Protein identity	Processes involved	Interaction with COP1	Literature source
HY5	AT5G11260	bZIP transcription factor	Photomorphogenesis, light signaling	Yes	Osterlund et al., 2000 Saijo et al., 2003
HYH	AT3G17609	bZIP transcription factor	Photomorphogenesis, light signaling	Yes	Holm et al., 2001
LAF1	AT4G25560	MYB transcription factor	Photomorphogenesis, light signaling	Yes	Seo et al., 2003
HFR1	AT1G02340	bHLH transcription factor	Photomorphogenesis, light signaling	Yes	Jang et al., 2005
BBX4/COL3	AT2G24790	B-box Zinc Finger protein	Photomorphogenesis, light signaling	Yes	Datta et al., 2006
BBX20/BZS1	At4G39070	B-box Zinc Finger protein	Photomorphogenesis, light signaling; BR signalling	Yes	Fan et al., 2012
BBX22/LZF1/ STH3	AT1G78600	B-box Zinc Finger protein	Photomorphogenesis, light signaling	Through HY5?	Datta et al., 2008
BBX24/STO	AT1G06040	B-box Zinc Finger protein	Photomorphogenesis, light signaling	Yes	Holm et al., 2002
PIL1	At2g46970	bHLH transcriptional factor	Photomorphogenesis, light signaling	Yes	Luo et al., 2014
GATA2	AT2G45050	GATA transcription factor	Photomorphogenesis, light and BR crosstalk	Yes	Luo et al., 2010
phyA	AT1G09570	Phytochrome	Light perception	Yes	Seo et al., 2004
phyB	AT2G18790	Phytochrome	Light perception	Yes	Jang et al., 2010
BBX1/CO	AT5G15840	B-box Zinc Finger protein	Flowering	Yes	Jang et al., 2008 Liu et al., 2008
GI	AT1G22770	Unknown protein	Circadian rhythm and flowering	Through ELF3	Yu et al., 2008
SCAR1	AT2G34150	Root growth	Root growth	Yes	Dyachok et al., 2011
HRT	AT5G43470	R protein	Plant defense	Yes	Jeong et al., 2010
MYC2	AT1G32640	bHLH transcriptional factor	JA signaling	Yes	Chico et al., 2014

Adapted from (Lau and Deng, 2012) with minor modification.

2. SCIENTIFIC AIMS

Overall Aim

The general aim of this thesis was to increase our knowledge about regulatory mechanisms of light-regulated plant development.

Specific Aims

●To investigate the role of B-box protein, BBX21, in the crosstalk between light and ABA signaling during seed germination (**Paper I)**.

●To identify and characterize novel regulators of COP1 or COP1-regulated development **(Paper II and III)**.

3. RESULTS AND DISCUSSION

B-box proteins which contain one or two B-box domains involved in many light-regulated developmental processes. B-box protein, BBX21/STH2, genetically and physically interacts with HY5 and participates in the crosstalk between the light- and ABA signaling networks. The first part of this doctoral work was to characterize the role of BBX21 in the crosstalk between light and ABA networks and how BBX21 together with HY5 and ABI5 integrated these two signals at the molecular level. COP1 is a key repressor of light signaling in plants. Though the function of COP1 is well studied, the regulatory mechanism on COP1 remains poorly understood. The second part of this doctoral work was aimed to identify and characterize novel COP1 regulators and/or COP1-regulated factors to further elucidate the regulatory mechanisms on COP1 and/or the COP1-controlled processes. Results from these studies can be found in **Paper I, II, III**. Here I discuss the major findings and implications from these papers.

3.1 B-box Proteins in the Crosstalk between Light and Hormone Signaling

Ring-finger domain (originally termed an A-box) is the first identified Zn-binding domain, and the B-box domain is first identified in animal protein as a second Zn-binding domain. In *Arabidopsis,* there are 32 B-box proteins, which are grouped into five subfamily according to the structure of protein domain (Khanna et al., 2009). A range of BBX proteins participate in the light and various hormone transduction networks (Gangappa and Botto, 2014). BBX4/COL3, BBX20/BZS1, BBX21/STH2 and BBX22/STH3 act as positive regulators of light signaling (Datta et al., 2006, 2007, 2008; Fan et al., 2012), whereas BBX16/COL7, BBX18/DDB1a, BBX24/STO, BBX25/STH, and BBX32 negatively regulate plant photomorphogenesis (Holtan et al., 2011; Wang et al., 2011a; Gangappa et al., 2013; Wang et al., 2013). BBX21 interacts with both HY5 and COP1 to positively regulate light-dependent development (Datta et al., 2007). In ABA signaling pathway, two bZIP transcription factor, HY5 and ABI5 play positive roles, since loss of either HY5 or ABI5 results in low sensitivity to ABA in seed germination and root development. HY5 directly binds to the promotor of *ABI5* to activate its expression and *ABI5*-regulated gene expression (Chen et al., 2008). In **Paper I** ABI5 was shown to be able to directly bind to G-boxes on its own promoter to activate its own expression. BBX21 physically associated and formed inactive heterodimers with HY5 and ABI5. *ABI5* or *ABI5*-regulated gene expression was

down-regulated due to the repression of HY5 and ABI5 activity. Therefore, BBX21 negatively mediated ABA responses by suppressing HY5 and ABI5 activity (**Paper I**). It has been shown that BBX4 and BBX22 interact with HY5 to activate transcription of *HY5* (Datta et al., 2006, 2008), whereas BBX24, BBX25, and BBX32 associate with HY5 to repress the transcriptional activity of *HY5* (Holtan et al., 2011; Gangappa et al., 2013). Interestingly, based on our data and those reported in previous studies, HY5 appears to play a central role in the BBX-mediated transduction network. It is proposed that BBX proteins form inactive heterodimers with other transcriptional activators and act as transcriptional repressors in response to light and various hormone signals (Gangappa et al., 2013; Gangappa and Botto, 2014). The characterization of the role of BBX21 in the light and ABA network further supported this hypothesis (**Paper I).**

3.2 *cop1* Suppressors

Weak alleles of *cop1* such as *cop1-4*, *cop1-6* display constitutive photomorphogenic phenotype in the dark (McNellis et al., 1994a). Mutations in several BBX proteins including *BBX4*, *BBX20, BBX21*, and *BBX22* partially suppress *cop1* phenotype in the dark. These BBX proteins are proposed to act as downstream substrates of COP1 and their protein stability are regulated in a COP1-dependent manner (Datta et al., 2006, 2007, 2008; Fan et al., 2012). The recessive locus, *hy5,* is able to completely suppress *cop1* in darkness and is hyposensitive to various light (white, red , far-red and blue) (Ang and Deng, 1994). The dominant *cop1* suppressor, *fin219,* is specifically insensitive to far-red light and hypocotyl length of *fin219 cop1-6* double mutant seedlings is indistinguishable with that of WT in the dark (Hsieh et al., 2000). Both *hy5* and *fin219* are identified and characterized via a genetic screen. Further genetic and biochemical studies demonstrate that HY5 and FIN219 are two key two regulators of light signalling and involved in COP1-mediated developmental pathway. Being a downstream substrate of COP1, HY5 is regulated by COP1 via its E3 ubiquitin ligase activity (except UV-B signaling). The COP1-HY5 regulon represents a key model in the plant photomorphogenic development. FIN219 which is involved in phyA-mediated pathway, might regulate the COP1 nucleocytoplasmic partitioning in response to far-red light (Wang et al., 2011b). Thus, it will be worth to identify and characterize more novel *cop1* suppressors, since it will offer us more insights regarding to COP1 regulated pathway and/or regulatory mechanism on COP1. By a forward genetic screen, two novel recessive *cop1* suppressors were identified and characterized, namely *csu1* and *csu2* (**Paper II and III**). Either *csu1* or *csu2* alone completely suppressed

cop1-6 in the dark and partially in the light. *hy5* together with *csu1* or *csu2* completely rescued the dramatically short hypocotyls of *cop1-6* to that of WT levels in the light. These genetic data indicate that the normal accumulation of these proteins contribute to the constitutive photomorphogenic phenotype of *cop1* in the dark. Loss of CSU1 resulted in hypersensitivity to various light conditions tested (white, red, far-red and blue), indicating CSU1 plays a negative role in light inhibition of hypocotyl growth. While mutations in *CSU2* leaded to dramatically short primary root length compared to that of WT in the light, implying CSU2 positively controls root elongation in response to light.

3.3 Regulatory Mechanism on COP1

The E3 ubiquitin ligase COP1 plays a central role during the plant development and mediates a number of developmental processes, such as photomorphogenesis, flowering, root development, pigment accumulation (Lau and Deng, 2012; Huang et al., 2014). The COP1 and its substrates like HY5 abundance *in vivo* quantitatively correlates with the level of plants photomorphogenesis, and their appropriate protein level are essential for the optimal development of plants (McNellis et al., 1994b; Stoop-Myer et al., 1999; Osterlund et al., 2000). Therefore, the tight control of COP1 activity or abundance is quite essential to ensure the normal abundance of COP1 targets and optimize the development of plants. Previous and our studies demonstrate that regulation on COP1 is a fine-tuning mechanism through multiple layers including promoting the translocation of COP1 from the nucleus to the cytoplasm, ubiquitination and degradation of COP1, repressing and enhancing COP1 activity.

3.3.1 COP1 Nucleocytoplasmic Partitioning

Light is the first factor that is identified to regulate COP1 translocation and activity. In darkness, COP1 accumulates in the nucleus and forms nuclear speckles where it targets a number of proteins for ubiquitination via its E3 ubiquitin ligase activity. Upon light exposure, COP1 migrates to the cytoplasm and is inactivated, allowing accumulation of COP1 target proteins in the nucleus to promote photomorphogenesis (von Arnim and Deng, 1994). Due to the slow kinetics of the nuclear exclusion of GUS-COP1 (approximately 24 hours), this strategy is regard as a long-term suppression of COP1 under extended light conditions (Arnim and Deng, 1994; Arnim et al., 1997). However, a recent study challenges this classic view. Upon light illumination, the half life of the transportation of YFP-COP1 out of the nucleus is 2.5±0.5 hours and is

sufficiently rapid to contribute to the early stabilization of YFP-HY5 (half life 2.9 ±0.2 hours) by repressing COP1 activity (Pacín et al., 2013; 2014). Low temperature and heat shock are also two environmental factors that regulate COP1 nucleocytoplasmic partitioning. Either low temperature or heat shock stimuli promotes COP1 export from the nucleus to the cytoplasm in the dark, thereby allowing the accumulation of its target proteins like HY5 (Catalá et al., 2011; Karayekov et al., 2013). While the plant phytohormone ethylene enhances the movement of COP1 to the nucleus to degrade downstream substrates such as HY5 in the light, thus to promote hypocotyl growth (Yu et al., 2013). phyA, phyB, CRY1, FIN219 and CSN1 are also involved in the regulation of COP1 nucleocytoplasmic partitioning (Osterlund and Deng, 1998; Wang et al., 2009; Wang et al., 2011b). Therefore, it can be hypothesized that COP1 translocation between the nucleus and the cytoplasm may represent a key regulatory mechanism to modulate COP1 activity in response to various external or internal factors (Figure 5).

3.3.2 Regulators of COP1

COP1 directly functions downstream of photoreceptors, and its activity is also regulated by these photoreceptors. Red and far-red light receptors down-regulate COP1, though the mechanism by which phytochromes regulate COP1 activity remains uncertain (Tepperman et al., 2001, 2004). In response to blue light, CRY1 and CRY2 interact with SPA1 to repress COP1 activity. CRY1-SPA1 interaction disrupts COP1-SPA1 complexes which form in the dark, while CRY2-SPA1 association enhances the CRY2-COP1 interaction (Lian et al., 2011; Liu et al., 2011; Zuo et al., 2011).

Though COP1 is an E3 ubiquitin ligase, itself is also ubiquitinated and proteasomally regulated, since both COP1 and its mutant with four cysteine residues substitution (C52S, C55S, C86S, C89A) in the Ring-finger domain were unstable and stabilized by the proteasome inhibitor MG132 *in vivo* in *Arabidopsis* (Seo et al., 2003, 2004). In darkness, another E3 ubiquitin ligase, CSU1, was recruited into nuclear speckles by COP1 for ubiquitination and degradation to maintain its homeostasis *in vivo* (Figure 5) **(Paper II)**. Considering that self-ubiquitination of COP1 *in vitro* and remaining ubiquitinated COP1 in *csu1* mutants *in vivo*, it is therefore possible that COP1 is at least in part responsible for its own abundance *in planta*. It is important to point out that CSU1 negatively regulated SPA1 as well, though it is not clear whether CSU1 directly targets SPA1 for ubiquitination. The negative regulation of SPA1 abundance by CUS1 may disrupt COP1-SPA1 complexes, which in

turn, repressing COP1 activity. Taken together, these results suggest that CSU1 may have a negative effect on the whole COP1-SPA1 complex in the dark.

In the dark, two COP1 and two SPA proteins form stable core complexes through their coiled-coil domains and contribute to ubiquitination and degradation of HY5 (Zhu et al., 2008). SPA1 enhances the COP1 activity on LAF1 at the low concentration of COP1 (Seo et al., 2003). PIF1 physically interacts with COP1, and promotes COP1 self-ubiquitination and poly-ubiquitination on HY5 (Xu et al., 2014). CSU2 interacted and co-localized with COP1 in nuclear speckles through their coiled-coil domains. While the CSU2-COP1 association repressed the COP1 E3 ubiquitin ligase activity towards HY5 (Figure 5) **(Paper III)**. The modulation of COP1 activity by SPA1, PIF1 or CSU2 may specifically occur in the nucleus in darkness. Multiple pieces of evidence support this hypothesis. 1) COP1 translocates into cytoplasm in the light; 2) Light activates photoreceptors like CRY1 and CRY2, ultimately disrupt COP1-SPA1 complex; 3) PIF1 is enriched in the dark, but unstable in the light; 4) CSU2 is constantly localized in the nucleus both in the light and dark. Consistently, COP1 specifically forms nuclear speckles where it targets its substrates for ubiquitination and represses the photomorphogenesis in darkness. Moreover, CSU1 ubiquitinated COP1 in the nuclear speckles in the dark and light terminated this modulation by triggering COP1 export from the nucleus **(Paper II)**. Together, it is evident that various effectors contribute to the fine-turning regulation on COP1 to tight control its appropriate abundance and/or activity, which ensure the normal development of plants in darkness (Figure 5).

3.4 COP1 nuclear bodies

In mammalian cells, a variety of components of the ubiquitin-proteasome pathway have been shown to form nuclear bodies. These sites are called clastosomes where proteolysis of many protein substrates is taking place (Lafarga et al., 2002). In *Arabidopsis*, phyA, phyB and CRY2 activated by light rapidly import into the nucleus and form photobodies (Yamaguchi et al., 1999; Kircher et al., 2002; Yu et al., 2009). phyA, PIF3 and FHY1 localize in the nuclear bodies prior to their degradation (Chen, 2008), implying that nuclear bodies in plants might be the sites for protein ubiquitination and degradation as well. COP1 nuclear bodies specifically forms in the dark, and light destroys these subnuclear foci (von Arnim and Deng, 1994) **(Paper II)**. COP1 coiled-coil domain is required and sufficient for the formation of nuclear bodies (Stacey and von Arnim, 1999). In the dark, COP1 co-localizes with a number of substrates including phyA, HY5, HYH, HFR1, LAF1, BBX4, BBX21, BBX22 as well as

its complex partner SPA1 in the nuclear bodies (Ang et al., 1998; Holm et al., 2002; Seo et al., 2003; Jang et al., 2005; Dattas et al., 2006, 2007, 2008). CSU1 and CSU2 uniformly localized throughout the nucleus, while were recruited by COP1 into nuclear bodies **(Paper II, III)**. In these sites, protein ubiquitination and modification may occur.

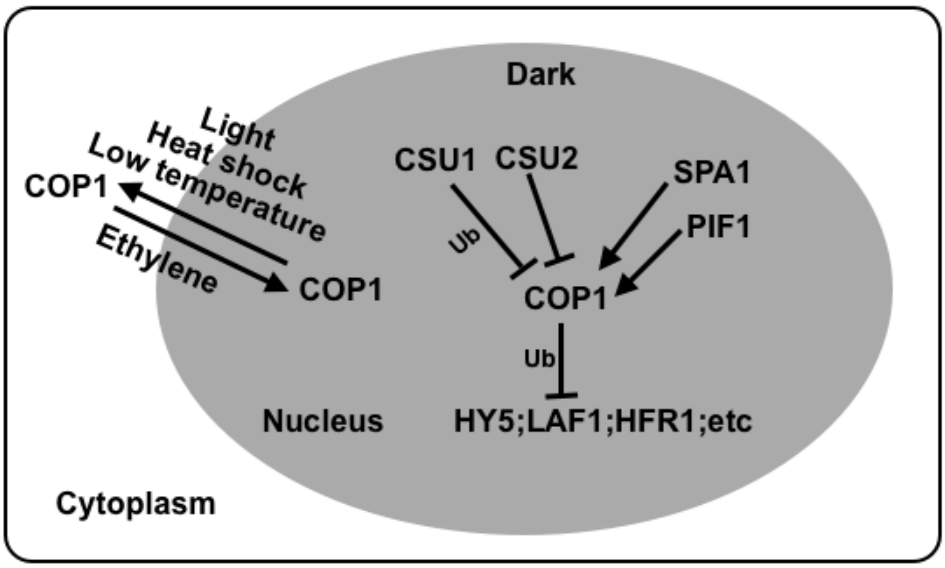

Figure 5. A proposed model of modulation on COP1.
In the dark, COP1 is enriched in the nucleus and targets downstream substrates such as HY5, LAF1, HFR1, for ubiquitination and degradation (Osterlund et al., 2000; Saijo et al., 2003; Seo et al., 2003; Jang et al., 2005). CSU1 specifically ubiquitinates COP1 to maintain its homeostasis, while CSU2 interacts with COP1 and represses its activity. SPA1 and PIF1 form heterodimers with COP1 to enhance its activity (Seo et al., 2003; Zhu et al., 2008; Xu et al., 2014). Light, heat shock, and low temperature promote COP1 export from the nucleus to the cytoplasm and inactivate COP1 activity (von Arnim and Deng 1994; Catalá et al., 2011; Karayekov et al., 2013). Plant hormone ethylene prevents movement of COP1 from the nucleus to the cytoplasm in the light (Yu et al., 2013). Ub represents ubiquitination.

4. CONCLUSIONS AND PERSPECTIVES

Light is a key environmental factor, which regulates many developmental processes throughout the life cycle of plants. Tremendous progress has been made in the field of plant photomorphogenesis and hormone signaling pathway in recent years. The positive regulator of light signaling, BBX21, functioned negatively in the light-mediated seed germination in response to ABA. BBX21 formed heterodimers with HY5 and ABI5, which in turn, repressing the transcriptional activation of *ABI5*. **(Paper I)**. BBX proteins mediate a range of light-regulated developmental processes as well as various hormone signaling networks. The biochemical mechanisms of BBX proteins involved in these developmental processes appear to operate at several different levels. 1) BBX proteins may function as transcription factors and directly bind to promoters of downstream target genes to activate or repress their gene expression; 2) BBX proteins act as transcriptional co-repressors by forming inactive heterodimers with other transcription factor proteins to inhibit their transcriptional activities; 3) BBX proteins together with other transcriptional activators to co-activate the expression of downstream target genes.

CSU1 acted as an E3 ubiquitin ligase, which co-localized with COP1 in nuclear speckles and targeted COP1 for ubiquitination to create a balance on COP1 levels inside the plant cells in darkness **(Paper II)**. *csu1* mutants seedlings grown in the light showed pale cotyledon phenotype, and a reduced chlorophyll level compared with WT seedlings (unpublished data). Several components of light signaling have been shown to participate in chlorophyll synthesis pathway. HY5 positively regulates chlorophyll synthesis, while PIF1 and PIF3 are repressors of chloroplast development (Holm et al, 2002; Huq et al., 2004; Stephenson et al., 2009). It would be interesting to explore the molecular role of CSU1 in the chlorophyll synthesis.

COP1 is found in a 700 KD complex and COP1-SPA complex is 440 KD in size (Zhu et al., 2008), implying other unknown components exist in COP1 complex. To identify and characterize these unknown components will help us further elucidate the role of this complex in the plants development. Two COP1 and two SPA form stable core complexes through their coiled-coil domains, while COP1 interacted and co-localized with CSU2 in the nucleus speckles through their coiled-coil domains **(Paper III)**. It will be of interest to test whether CSU2 is one of the components in the COP1 complex.

COP1 is able to migrate between the nucleus and the cytoplasm in response to the light or darkness. phyA, phyB and CRY1 have been shown to contribute to COP1 nucleocytoplasmic partitioning in response to light (Osterlund and Deng, 1998). However, how these photoreceptors regulate the COP1 translocation from the nucleus to the cytoplasm remains unclear. To further explore novel components responsible for the COP1 nucleocytoplasmic partitioning will provide more new insights into this fundamental regulatory mechanism.

Based on the current classic view, light inactivates COP1 by triggering it translocate from the nucleus to the cytoplasm (Arnim and Deng, 1994; Arnim et al., 1997; Pacín et al., 2013; 2014). If COP1 is inactivated in the cytoplasm in the light, *cop1* mutants should exhibit normal phenotype in the light. However, phenotypic analysis of *cop1* mutants reveal that *cop1* null alleles are adult lethal and *cop1* weak alleles develop short hypocotyls, high anthocyanin accumulation, short primary roots, dwarf adult phenotype in the light (Deng et al., 1991, 1992; McNellis et al., 1994a), indicating COP1 in the light possesses biological activity and mediates these light-controlled developmental processes. Biochemical assays demonstrate that COP1 forms nuclear speckles and acts as an E3 ubiquitin ligase in darkness. However, the exact molecular role of COP1 in the cytoplasm in the light need further investigation.

COP1 forms complex with other components like SPA and this complex in concert with CDD complex, COP9 signalsome, CUL4-DDB1 complex mediate the 26S proteasome pathway and participate in a variety of cellular and developmental processes (Huang et al., 2014). However, COP1 alone possesses E3 ubiquitin ligase activity, and SPA1, PIF1 or CSU2 are involved in the modulation of its activity (Zhu et al., 2008; Xu et al., 2014) **(Paper III)**. By using a combination of biochemistry, genetics, and structural biology approaches, it will help us gain more novel insights into mechanism by which COP1 together with these proteins synergistically regulate multiple developmental processes of plants.

Light activates photoreceptors and subsequently initiates photomorphogenesis. Transgenic *Arabidopsis* seedlings expressing gain-of-function phyA, phyB, CRY1, CRY2 or UVR8 mimic light-grown WT phenotype in the dark, suggesting that each of these constitutively active photoreceptors is sufficient to turn on photomorphogenesis in darkness (Yang et al., 2000; Wang et al., 2001; Su and Lagarias, 2007; Gu et al., 2012; Heijde et al., 2013). This is possible due to the suppression of COP/DET/FUS activity by these active

photoreceptor variants. Consistent with this notion, recessive *cop/det/fus* mutant seedlings display de-etiolated phenotype in the dark (Wei and Deng, 1996). SPAs are components in COP1 complexes and responsible for the COP1 activity (Zhu et al., 2008). PIFs act downstream of phytochromes and enhance the COP1 activity (Xu et al., 2014). Loss of either SPAs or PIFs show constitutive photomorphogenic phenotype in the dark as well (Laubinger et al., 2004; Leivar et al., 2008). These suggest that COP1 appear to play a central role in this complicated and delicate network. COP1-6 mutant is biologically active *in vivo*. CSU1 negatively mediated both COP1 and SPA1 at post-translational level. Mutation in CSU1 released this negative regulation, thereby allowing the accumulation of COP1-6 and SPA1 in *cop1-6*. *csu1 cop1-6* double mutant seedlings exhibited etiolated phenotype in the dark **(Paper II)**. CSU2 repressed COP1 activity *in vivo* and loss of CSU2 interrupted this suppression. Thus, *csu2 cop1-6* mutant seedlings developed skotomorphogenic phenotype in the dark **(Paper III)**. These findings further support the conclusion that COP1 is key repressor of light signaling and suppression of COP1 activity will turn on photomorphogenesis.

The COP/DET/FUS pathway is one of few examples of a regulatory system first identified in plants, and then found to control central processes also in animals and humans (Yi and Deng, 2005). Mammalian COP1 is recently shown to mediate in tumor development and functions as a critical negative regulator of tumor suppressor p53 and oncogene proteins c-Jun and ETS (Dornan et al., 2004; Migliorini et al., 2011; Vitari et al., 2011; Lu et al., 2014). In order to stabilize p53 and exert its tumor suppressor properties in response to DNA damage, the ataxia telangiectasia mutated (ATM) protein kinase phosphorylates COP1 on Ser-387, thereby initiating the self-ubiquitination of COP1 and subsequently to abrogates the ubiquitination and degradation of p53 (Dornan et al., 2006). In addition, 14-3-3σ targets phosphorylated COP1 for nuclear export, thereby preventing COP1-mediated p53 nuclear export (Su et al., 2010). We take advantage of the power of genetics to identify and characterize novel components and mechanisms that will provide novel and valuable insights into the molecular function of this evolutionary conserved pathway. This knowledge will further our understanding of light signaling in plants and will continue to guide studies of the mammalian COP1 and the COP/DET/FUS pathway in normal and tumor development. Database search and alignment analysis revealed that CSU1 and CSU2 were highly conserved from plants to animals as well (**Paper II and III**). Moreover, the ortholog of CSU1 in human, Nitric Oxide Synthase Interacting Protein (NOSIP)/mammalian Receptor-associated Ubiquitin Ligase (RUL), also possesses E3 ubiquitin ligase activity

and mediates the ubiquitination of Erythropoietin Peceptor (EpoR) (Friedman et al., 2003). Therefore, it will be interesting to test whether NOSIP/RUL contributes to the ubiqtuination of COP1 and participates in the tumor development. While CSU2 showed 34% amino acid sequence identity with its ortholog in human, *Homo sapiens* (NP_057037), of which the exact role remains to be explored. The identification and characterization of novel COP1 suppressors will provide a handle to further analyze this critical and conserved pathway.

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