On the interaction of light and auxin signalling in *Arabidopsis thaliana*

Inaugural-Dissertation

zur
Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Universität zu Köln

vorgelegt von

Sebastian Rolauffs

aus Willich (Kreis Viersen)

2012

Tag der mündlichen Prüfung: 22.06.2012

Berichterstatter/in:	Prof. Dr. Ute Höcker
	Prof. Dr. Martin Hülskamp
Prüfungsvorsitzender:	Prof. Dr. Wolfgang Werr
Beisitzerin:	Dr. Leonie Kokkelink

Content

Abbreviations

aa	amino acid
35S	35S promoter from Cauliflower Mosaic Virus
°C	degree Celsius
µg	microgram
µl	microlitre
µm	micrometre
B, Bc	blue light, continuous B
D	darkness
FR, FRc	far-red light, continuous FR
R, Rc	red light, continuous R
R:FR	ratio of red-light to far-red light fluences
HIR	high irradiance response
(b)HLH	(basic) helix-loop-helix
bp	base pair
BR	Brassinosteroids
cDNA	complementary DNA
cc	coiled-coil domain
cm	centimetre
Col-0	Columbia; ecotype of *Arabidopsis thaliana*
Ler	Landsberg *erecta*; ecotype of *Arabidopsis thaliana*
RLD	ecotype of *Arabidopsis thaliana*
WT	wild type
DNA	desoxyribonucleic acid
et al.	et alterni (Lat.) = and others
FL	full-length
GFP	green flourescent protein
YFP	yellow flourescent protein
h	hour
HA	protein tag (Influenza hemagglutinin)
kb	kilo bp (= 1000 bp)
kDa	kilo Dalton
LD	long day
M	molar (mol/l)
mg	milligram
mM	millimolar
min	minute
mRNA	messenger RNA
MS	Murashige and Skoog medium
nm	nanometre
p	promoter
PCR	polymerase chain reaction
Pfr	far red light absorbing conformation of the phytochromes
Phy	phytochrome
Pr	red light absorbing conformation of the phytochromes
RNA	ribonucleic acid
rpm	rounds per minute
RT-PCR	reverse transcription PCR
sec	second
LD	long days
SD	short days

SEM	standard error of the mean
NPA	1-N- naphthylphtalamic acid
T-DNA	transfer DNA
EMS	Ethylmethansulfonat (mutagen)
UTR	untranslated region of a transcript
UV	ultraviolet
LUC	luciferase (from firefly)
GUS	β-glucoronidase

Nomenclature:

SPA1	gene, locus, wild-type allele
spa1	mutant allele
SPA1	protein

Exception (Photoreceptors):

PHY	gene, locus, wild-type allele
phy	mutant allele
phy	holoprotein (with chromophor)

Zusammenfassung

Der COP1/SPA Komplex ist ein zentraler Regulator der Photomorphogenese in Arabidopsis. *COP1* wird für die Streckungsantwort des Hypokotyls bei einem niedrigen Verhältnis von Rotlicht zu Dunkelrotlicht (R:FR) benötigt, welches durch dichtstehende Nachbarn an natürlichen Standorten auftreten kann. In dieser Studie wurde gezeigt, dass *SPA* Gene essentiell für Streckungsantworten bei niedrigen R:FR Bedingungen sind. Von den *SPA* Genen waren vor allem *SPA1* und *SPA4* für die Keimlingsantworten zuständig. Nachbarn lösen auch eine Streckungsantwort der Blattstiele aus. Hierbei waren die *SPA* Gene und *COP1* essentiell. Die Blütenbildung wird durch niedrige R:FR Bedingungen beschleunigt und der COP1/SPA Komplex reguliert den Blühzeitpunkt im Kurztag. Es wurde gezeigt, dass weder *COP1* noch *SPA* Gene eine Funktion in der Beschleunigung der Blütenbildung in niedrigen R:FR Bedingungen haben. Eine Promotor-Tausch-Analyse von *SPA1* und *SPA2* in der Hypokotylstreckungsantwort auf niedrige R:FR Bedingungen enthüllte eine potentielle Funktion für SPA2 in diesem Prozess, allerdings nur, wenn SPA2 unter der Kontrolle des *SPA1* Promoters exprimiert wurde. Genetische Interaktionsstudien zeigten, dass *spa* Mutationen mit der *hfr1* Mutation in der Hypokotylstreckungsantwort bei niedrigen R:FR Bedingungen interagieren. Dies deutet darauf hin, dass eine Akkumulierung von HFR1 in den *spa* und *cop1* Mutanten zum Fehlen der Streckungsantwort beitragen könnte. Genetische Interaktionsstudien zeigten auch eine Interaktion von der *cop1* und der *phyB* Mutation und von *spa* Mutationen mit der *phyA* Mutation in der Hypokotylstreckungsantwort auf niedrige R:FR Bedingungen. Außerdem wurde bei einer gewebespezifischen Analyse eine Funktion von SPA1 in der Epidermis gefunden, während die Expression von SPA1 im Phloem auf die meisten Entwicklungen von Keimlingen dominiert. Darüber hinaus wurde gezeigt, dass eine Zunahme der Auxinsignaltransduktion in R:FR Bedingungen in *spa* Mutanten nicht stattfindet. Die Transkriptmengen von *YUC8,* einem Auxinbiosynthesegens, die im WT hochreguliert wurden, reagierten in den *spa* Mutanten nicht auf niedrige R:FR Bedingungen. Die Auxinsignaltransduktion war in *spa* Mutanten auch im Keimlingsstadium in Dunkelheit und monochromatischem Licht verändert und in Blättern, was darauf hindeuten könnte, dass eine veränderte Auxinsignaltransduktion zu den veränderten Keimlingsphänotypen und dem Zwergwachstum der *spa* Mutanten beitragen könnte. Es ist bekannte, dass eine Anzahl von auxin-induzierten Genen lichtreprimiert ist, aber, ob diese Lichtregulation direkt oder indirekt über Auxin funktioniert ist noch nicht geklärt. In diesem Zusammenhang konnten für zwei G-Box Motive des *IAA19* Promoters eine Beteiligung an der Lichtregulation von *IAA19* gezeigt

werden, während ein Auxinantwortselement (AuxRE), aber nicht die G-Box im *SAUR-AC1-l* Promotor zur Lichtregulation des *SAUR-AC1-l* Genes beitrug. Dies befürwortet die Ansicht, dass die Lichtsignaltransduktion direkt die Promotoraktivität von Zielgenen beeinflussen kann, aber auch über die Manipulation von Hormonsignalwegen Gene reguliert.

Abstract

The COP1/SPA complex is a central regulator of photomorphogenesis in Arabidopsis. *COP1* is required for the elongation response of the hypocotyl to a low red light to far-red light ratio (R:FR ratio), which is caused by close neighbours in natural habitats. In this study, it was shown that *SPA* genes were also essential for elongation responses to low R:FR conditions. *SPA1* and *SPA4* were the main *SPA* genes that regulate the responses of seedlings. Close neighbours also trigger an elongation response of leaf petioles. Here, the *SPA* genes and *COP1* were essential. Flowering is accelerated by low R:FR conditions and the COP1/SPA complex is a regulator of flowering time in SD. It was shown that neither *COP1* nor *SPA* genes had a function in the acceleration of flowering in response to low R:FR conditions. A promoter-swap analysis of *SPA1* and *SPA2* revealed a function for SPA2 in the elongation response of the hypocotyl to simulated shade, but only when expressed from the *SPA1* promoter. Furthermore, genetic interaction studies showed that *spa* mutations interacted with the *hfr1* mutation in the elongation response of the hypocotyl to low R:FR, indicating that over-accumulation of HFR1 may contribute to the lack of elongation response of *spa* and *cop1* mutants. Genetic interaction studies also revealed a genetic interaction of the *cop1* mutation with the *phyB* mutation and of *spa* mutations with the *phyA* mutation in the hypocotyl elongation response to low R:FR. Moreover, a tissue-specific function for SPA1 in the elongation response to low R:FR was found in the epidermis, while seedling growth in darkness and light was largely controlled by expression of SPA1 in the phloem. Furthermore, it was shown that the increase of the auxin signalling by low R:FR conditions was absent from two *spa* mutants and that the transcript levels of *YUC8*, an auxin biosynthesis gene, were unresponsive to low R:FR in a *spa* mutant, while up-regulated in the WT. Moreover, auxin signalling was found to be altered in *spa* mutants at the seedling stage in darkness and light and in adult leaves, which suggests that altered auxin signalling may contribute to the aberrant seedling phenotype and dwarfed growth of *spa* mutants. It is known that a number of auxin-induced genes are light-repressed, but whether the light-regulation is indirect via auxin or direct is not fully resolved. Here, two G-Box core motifs of the *IAA19* promoter were shown to contribute to the light-regulation of the *IAA19* gene, while an auxin response element (AuxRE), but not the G-Box present in the *SAUR-AC1-l* promoter was contributing to the light-regulation of the *SAUR-AC1-l* gene. This supports the notion that light signalling can directly act on promoter activity of target genes, but can also regulate genes via manipulation of hormonal pathways.

I. Introduction

I.1 Light perception and photomorphogenesis in *Arabidopsis thaliana*

Plants vitally depend on the energy they receive from the sunlight. The electromagnetic spectrum of the light that they can absorb to fuel their photosynthesis is referred to as the *photosynthetically active radiation* (PAR; 400-700 nm wavelengths). Beyond their energy consume, plants utilize light as a source of information about the environment they inhabit. They have developed the capacity to measure the wavelength composition, direction and duration. Light signals are used for the adaptation of germination, seedling and adult plant development and the transition to flowering to the environmental conditions in order to optimise reproductive success.

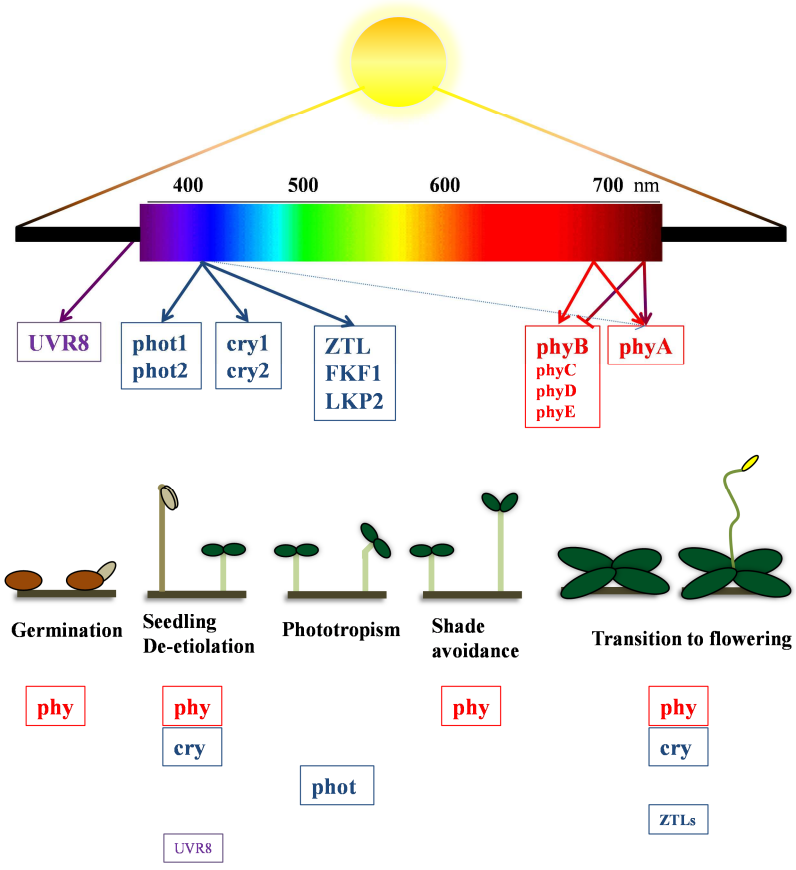

Figure I-1: The photoreceptors of *Arabidopsis thaliana* and their functions in plant development. UV-B light activates the ULTRAVIOLET RESISTANCE 8 (UVR8) receptor. UV-A and blue (B) light are perceived by the phototropins (phot1-2) and the cryptochromes (cry1-2). B light also activates the ZEITLUPE (ZTL) family of photoreceptors. The red (R) and far-red (FR) light spectrum is perceived by phytochromes (phyA-E). The phytochromes are involved in germination, seedling-deetiolation, shade avoidance and the transition from vegetative to reproductive growth. The cryptochromes also act on seedling-deetiolation and the transition to flowering. The Phototropins control growth towards or away from a light source (phototropism), while ZTL factors are involved in flowering time control and the UVR8-receptor contributes to photomorphogenic responses in seedlings.

Light perception is mediated by an array of photoreceptors that can be grouped into five main classes in *Arabidopsis thaliana*. These consist of five PHYTOCHROMES (phyA-E) that predominantly absorb in the red (R) and far-red (FR) light spectrum; two CRYPTOCHROMES (cry1 and cry2) and the two PHOTOTROPINS (phot1 and phot2) that perceive ultraviolet A (UV-A) and blue (B) light and the ZEITLUPE protein family (ZEITLUPE / FLAVIN-BINDING, KELCH REPEAT, F-BOX / LOV KELCH REPEAT PROTEIN 2 (ZTL/FKF1/LKP2)) that also absorbs blue light (B) (Briggs and Christie, 2002; Clack et al., 1994; Huala et al., 1997; Lin, 2002; Nelson et al., 2000; Somers et al., 2000). Furthermore, the recently identified ULTRAVIOLET RESISTANCE LOCUS 8 (UVR8) receptor is activated by UV-B light (Christie et al., 2012; Rizzini et al., 2011). A selection of important developmental processes regulated by the photoreceptors is shown in figure 1-1. First, the germination of seeds is induced by light in a red light-dependent manner by the phytochromes (reviewed in Franklin and Quail, 2010). Seedlings that grow in the absence of light depend on their seed storage of energy and biomolecules. They display long hypocotyls, closed apical hooks and closed, pale cotyledons. This skotomorphogenesis is continued until light is perceived. Once the seedlings reach the light, they undergo de-etiolation, which is characterised by the inhibition of the hypocotyl elongation, the opening of the cotyledons and the apical hook and the development of green chloroplasts. Morphological changes triggered by light are called photomorphogenesis. The de-etiolation of seedlings is driven by actions of phytochromes and cryptochromes (Strasser et al., 2010). The transition from the vegetative to the reproductive growth is regulated by light through the photoperiodic pathway, which involves the function of phytochromes, chryptochromes and the zeitlupe protein family (Yanovsky and Kay, 2002; Valverde et al., 2004; Somers et al., 2004). The floral inducer CONSTANS (CO) acts downstream of the photoreceptors in the photoperiodic pathway and is stabilized by long photoperiods that favour flowering (Laubinger et al., 2006; Valvere et al., 2004). CO promotes the expression of the floral integrators FLOWERING LOCUS T (FT) and of TWIN SISTER OF FT (TSF), which move to the shoot apical meristem (SAM) to promote the induction of flowering (Corbesier et al., 2007; Wenkel et al., 2006; Yamaguchi et al., 2005).

Besides the adaptations to the abiotic environment, plants have also developed the capacity to extract information about surrounding competitors from the ambient light, which represent a threat, as they might eventually shade the plants. Close neighbours can be detected well before they outgrow the plants as they reflect more FR light photons than any other component of the sunlight, because FR light is not used for photosynthesis and hardly

absorbed (reviewed in Vandenbussche et al., 2005). The phytochromes are capable of detecting this drop of the red light to far-red light (R:FR) ratio of the ambient light, and subsequently induce an increase in elongation processes, resulting in a longer hypocotyl, extended internodes and elongated petioles compared with plants grown in open sunlight (Ballare, 1999). Furthermore, the low R:FR conditions trigger an increase of the leaf angles (hyponasty), reduction of the leaf blade area, a lower branching of the shoot and an acceleration of flowering (Ballare, 1999). These reactions are collectively referred to as the shade avoidance syndrome (SAS). However, faster growth towards the sunlight to outcompete close neighbours is itself energy-consuming and may only be successful in certain environments, such as open fields, but futile in others (Yanovsky et al., 1995). Thus, plants have developed mechanisms to react appropriately to the different stages of canopy shading. Figure 1-2 illustrates the main stages of shade and the adaptations in Arabidopsis plants that detect competitors.

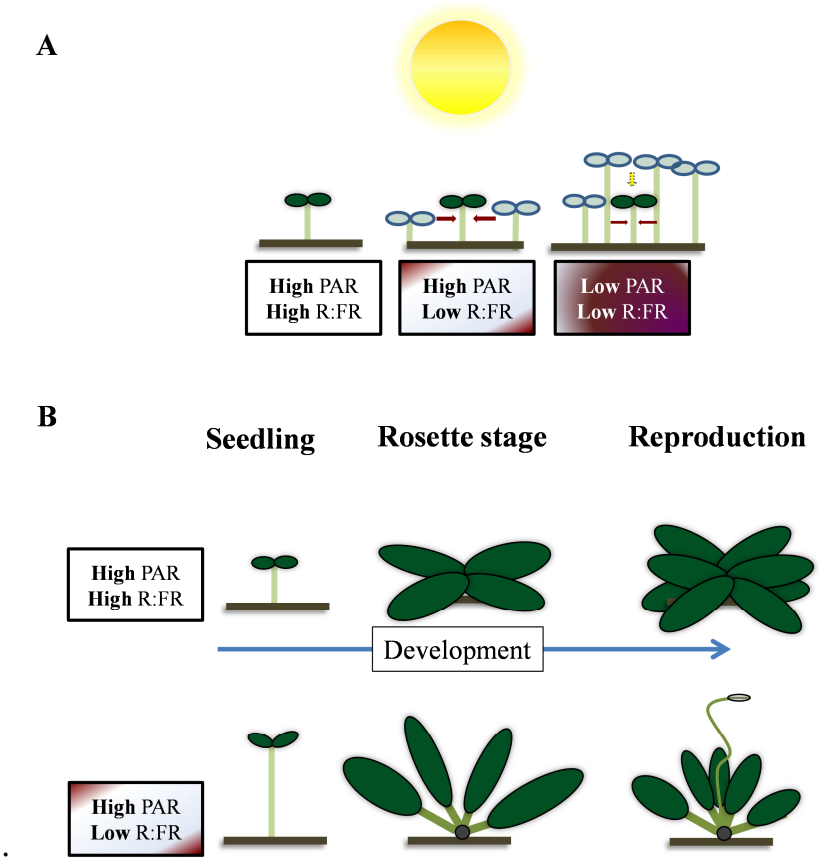

Figure I-2: Shade avoidance responses of *Arabidopsis thaliana*. A) Plants monitor the R:FR ratio of the ambient light to detect neighbours. Overgrown plants suffer from a lower photosynthetically active radiation (PAR). **B)** A low R:FR ratio of the ambient light triggers several elongation responses and leads to the acceleration of flowering.

I.1.1 Red and far-red light perception by the phytochromes

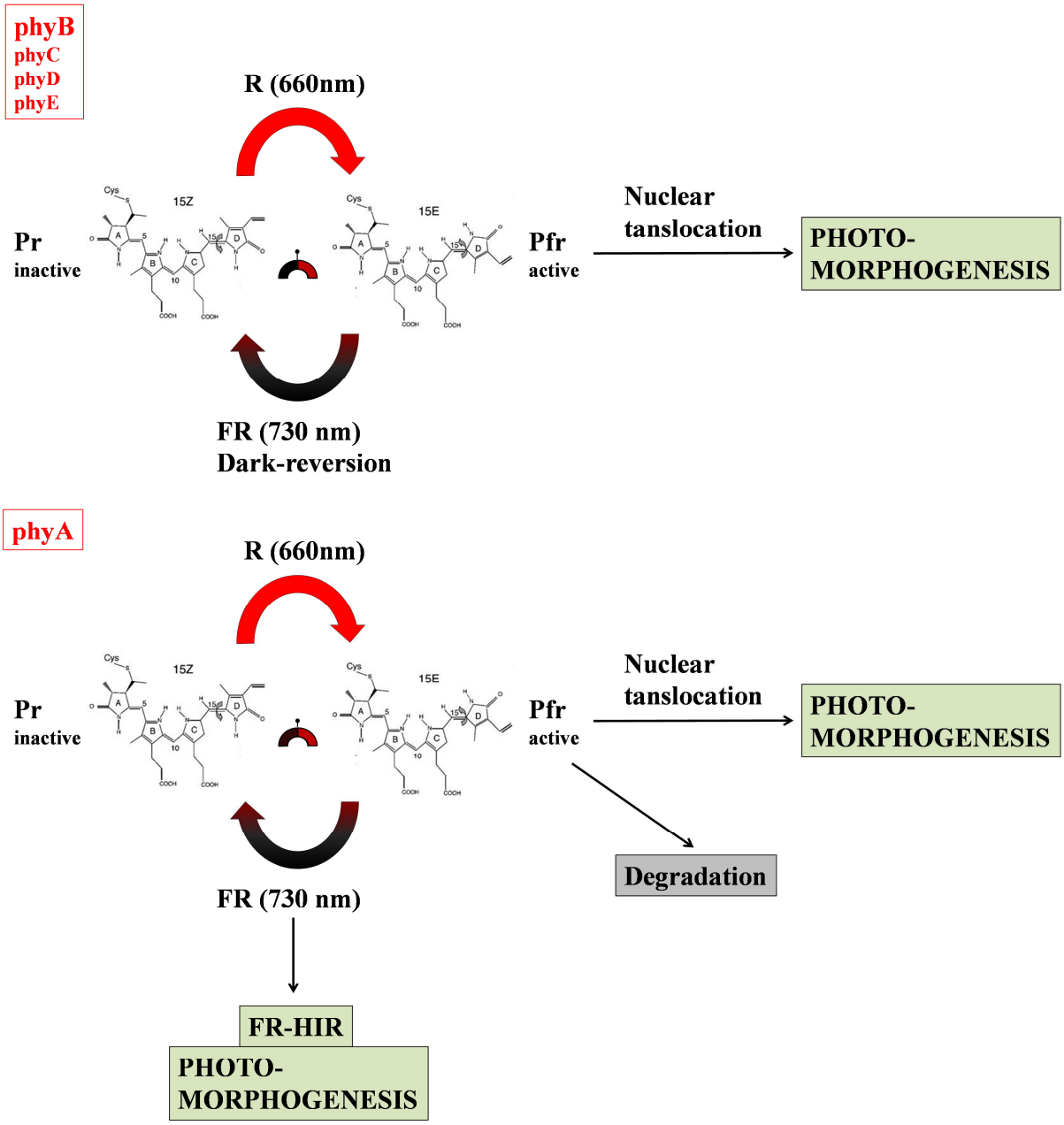

Figure I-3: Phytochromes perceive light of the red and the far-red light spectrum. Phytochromes are synthesised in the inactive Pr form that absorbs red light. The Pfr form is active and moves to the nucleus and cause photomorphogenesis. The Pfr form is transferred to the Pr form by FR light or by a light-independent mechanism called dark-reversion. PhyA additionally functions as a FR light receptor in the FR high irradiance response. The Pfr form of phyA is rapidly degraded after activation by red light.

The reactions to R and FR light and to different R:FR ratios are solely mediated by the phytochromes and depend on their unique properties and antagonistic activities. Each phytochrome consists of a 125 kDa polypeptide that carries a linear tetra-pyrrole chromophore (reviewed in Quail et al., 1995; Davis et al., 1999). Phytochromes act as dimers

and both, homo- and hero-dimerisation has been observed within the phytochrome family (Sharrock and Clack, 2004). The phytochromes can be subdivided into two groups according to their stability in the light. In Arabidopsis, the phytochrome type I consists only of phyA, which accumulates in darkness and is highly photo-labile. The phytochrome type II is formed by the remaining phyB, phyC, phyD and phyE, which are more stable in the light, but have also been shown to be regulated by protein degradation (Sharrock et al., 2002; Jang et al., 2010). One of the most striking features of the phytochromes is their function in R/FR reversible responses that are known as the low fluence responses (LFR; reviewed in Nagy and Schäfer, 2002). Upon absorption of red light (around 660 nm wavelength), the tetra-pyrrole chromophore changes its conformation and this leads to the activation of the phytochrome, which is transferred to its FR light-absorbing form (Pfr). The absorption of FR photons (around 730 nm wavelength) transfers the phytochrome back to the inactive R light-absorbing form (Pr). The cycling between the Pr form and the Pfr form creates a dynamic equilibrium of the phytochromes (photoequilibrium) that is rapidly altered in response to different light conditions (Nagy and Schäfer, 2002). Figure I-3 illustrates the R/FR reversibility of the phytochromes. It follows from the above that in low R:FR conditions, the photoequilibrium of the phytochromes is shifted towards the inactive Pr state. The inactivation of phyB is considered as one of the major functions of the phytochromes in neighbour detection, as *phyB* mutants display a constitutive shade avoidance phenotype even in open sunlight conditions and display only weak responses to low R:FR treatment (Halliday et al., 1994; Reed et al., 1993). Two additional phytochromes, phyD and phyE contribute to the shade avoidance responses, presumably employing the same molecular mechanism as phyB (Devlin et al., 2003).

In contrast to the type II phytochromes, phyA acts as a FR light receptor in the high irradiance response to FR light (FR-HIR; Nagy and Schäfer, 2002). Furthermore, phyA senses very low fluences of light (very low fluence response, VLFR) due to the high levels of phyA that accumulate in dark-grown seedlings and promotes germination (Botto et al., 1996). The photoconversion of phyA is similar to phyB, but as the phyA Pfr form is rapidly degraded, the phyA levels decrease strongly upon B and R light absorption (Sharrock et al., 2002). Therefore, phyA activity is negligible in direct sunlight. Moreover, phyA shows a maximum of activity in FR light (Nagy and Schäfer, 2002). Therefore, phyA activity is increased in low R:FR conditions. In deeper canopy shade, which is characterised by an additional loss in PAR, phyA is further stabilised by the low light intensities, which again increases its activity (Smith et al., 1997). Overall, phyA contributes to the shade avoidance responses especially at

lower light intensities and at very low R:FR conditions and counteracts the low R:FR-dependent inactivation of phyB (Smith et al., 1997). The phyA inhibition of elongation responses in deep shade conditions is relevant for the fitness of plants, because phyA mutants die in deep shade. Thus, phyA function is adaptive in deep shade, presumably because elongation in deep shade situations is too energy consuming for the seedlings (Yanovsky et al., 1995).

After activation by R light, the Pfr form of the phytochromes is imported to the nucleus by transport facilitators, which has long been established for phyA, but has recently also been shown for phyB and proposed to represent a general transport mechanism for phytochromes (Hiltbrunner et al., 2005 and 2006; Kircher et al., 1999; Pfeiffer et al., 2012). The nuclear localisation is crucial for phy function and results in a drastic change in the transcriptome (Huq et al., 2003; Tepperman et al., 2001).

I.2 Light signalling downstream of the photoreceptors

I.2.1 The PHYTOCHROME INTERACTING FACTORS (PIFs) promote skotomorphogenesis and shade avoidance responses

In darkness, the PHYTOCHROME INTERACTING FACTORS (PIFs) that belong to the basic HELIX-LOOP-HELIX (bHLH) transcription factor family are important to maintain skotomorphogenesis and the associated elongation responses (Martínez-García et al., 2000; Duek and Fankhauser, 2005). Hence, a *pif1 pif3 pif4 pif5* quadruple mutant (*pifq*) exhibits constitutive photomorphogenesis in darkness (Leivar et al., 2009). Furthermore, the transcriptome of dark-grown *pifq* mutants largely resembles the transcriptome of light-grown WT seedlings, which shows that the PIFs are important factors of gene expression in darkness (Leivar et al., 2009; Shin et al., 2009). PIF proteins bind to specific promoter sequences, *G-Boxes* that have a CACGTG core consensus motif and induce the expression of genes that mainly promote cell elongation (de Lucas et al., 2008; Huq et al., 2004; Martínez-García et al., 2000; Moon et al., 2008). G-Boxes are a member of the E-Box motif family that share a CANNTG consensus and are targeted by bHLH transcription factors in eukaryotes (Atchley and Fitch, 1997). Upon light perception, the phytochromes are transported to the nucleus and interact with the PIFs (Duek and Fankhauser, 2005; Hiltbrunner et al., 2005 and 2006; Kircher et al., 1999; Pfeiffer et al., 2012). The interaction with the phytochromes leads to the phosphorylation of the PIFs and their subsequent ubiquitination and degradation via the 26-S proteasome (Bauer et al., 2004; Huq et al., 2004; Lorrain et al., 2008). Thus, light negatively

acts on PIF protein levels and inhibits the expression of genes that are up-regulated by the PIFs (see fig. I-4). However, low R:FR conditions promote PIF protein levels and enhance the expression of elongation promoting genes that are under the control of PIFs (Martínez-García et al., 2000; Li et al., 2012; Lorrain et al., 2008). Three members of the PIF family, PIF4, PIF5 and PIF7, were shown to be involved in the elongation response of hypocotyls in response to low R:FR conditions (Li et al., 2012; Lorrain et al., 2008). As a *pif4pif5* double mutant and a PIF5 over-expresser both display a reduced elongation response of the hypocotyl to low R:FR compared with the WT, balanced PIF4 and PIF5 levels are thought to be required for the elongation of the hypocotyl in low R:FR. Furthermore, PIF4 and PIF5 negatively regulate phyB level, further promoting their own activity and shade reactions (Leivar et al., 2008). PIF4 and PIF5 act directly by binding to promoter elements in shade avoidance up-regulated genes.

I.2.2 The COP1/SPA complex is a central repressor of transcription factors

Besides the PIF transcription factors, additional proteins act downstream of the photoreceptors. A number of genes that promote skotomorphogenesis and inhibit photomorphogenesis were identified through the analysis of mutants that exhibit constitutive photomorphogenic development in darkness. These genes were unified in the *CONSTITUTIVE PHOTOMORPHOGENESIS (COP)*/ *DE-ETIOLATED (DET)*/ *FUSCA (FUS)* gene group (Wei and Deng, 1996).

CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) is a member of the COP/DET/FUSCA family that was shown to function as an E3 ubiquitin-ligase that acts negatively on photomorphogenesis by targeting transcription factors for degradation that promote light signalling (Deng et al., 1991; reviewed in Hoecker, 2005). It contains a WD40-domain and a coiled-coil domain that serve as protein-protein interaction domains and a RING finger domain, which is central for the E3-Ubiquitin-ligase function (Deng et al., 1992). Multiple mutants of the *suppressor of phyA-105-1* (*spa1*) and *spa1-like* (*spa*) mutations exhibit constitutive photomorphogenic phenotypes similar to the *cop1* mutants. This indicates that the SPA proteins are also important suppressors of photomorphogenesis. The *SPA1* gene was initially identified in a screen for suppressors of a weak *phyA* mutant and isolated by positional cloning (Hoecker et al., 1998 and 1999). *SPA1* counteracts phytochrome meditated inhibition of hypocotyl elongation (Parks et al., 2001). Three additional *SPA1-LIKE* genes (*SPA2-4*) have been uncovered in the Arabidopsis genome (Laubinger and Hoecker, 2003). The four *SPA* genes have overlapping, but also distinct functions in the regulation of light-

mediated plant development (Laubinger and Hoecker, 2003; Laubinger et al., 2004; Fittinghoff et al., 2006; Balcerowicz et al., 2010). In dark-grown seedlings, *SPA1* and *SPA2* are the main contributors to the repression of photomorphogenesis, while overstimulation of photomorphogenesis in the light is mainly repressed by *SPA1*, *SPA3* and *SPA4* (Laubinger et al., 2004). Adult plant development and final leaf size is mainly controlled by *SPA3* and *SPA4* (Laubinger et al., 2004; Fackendahl, PhD Thesis, 2011). Flowering time in short days (SD) depends on functional *SPA1*, as a *spa1* single mutant flowers early in short days (Laubinger et al., 2006). The SPA proteins contain a C-terminal WD40 domain and a central coiled-coil (cc) domain, which they both share with the COP1 protein and a more diverse N-terminal domain (Hoecker et al., 1999). The cc-domain is necessary for SPA-SPA interaction (Hoecker and Quail, 2001; Zhu et al., 2008). The N-terminus contains a region that is reminiscent of a kinase-domain, though a kinase function of the SPA proteins has never been shown (Hoecker et al., 1999; Hoecker, 2005).

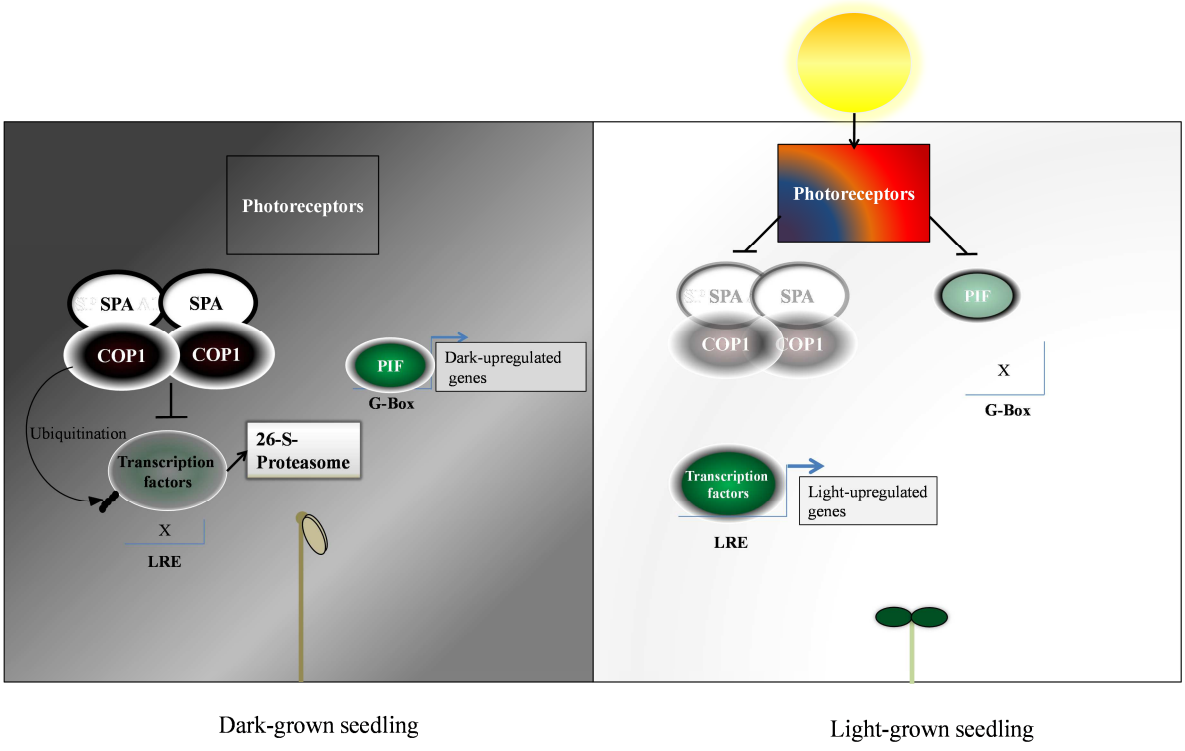

Figure I-4: COP1/SPA and PIF function in transcriptional control of light regulated genes downstream of the photoreceptors. In darkness, the COP1/SPA complex is active and targets transcription factors for degradation by ubiquitination. Additionally, PIF proteins bind to PIF-binding sites (G-boxes) of target genes and promote the expression of dark-up-regulated genes. These conditions lead to skotomophogenesis. The photoreceptors are activated by light and inhibit the COP1/SPA complex. The transcription factors accumulate and light regulated genes are expressed. Furthermore, PIF proteins are inhibited by the phytochromes and subsequently degraded, which inhibits the expression of dark-up-regulated genes. This causes photomorphogenesis.

It was shown that COP1 and SPA interact via their cc-domains and act together in a tetrameric complex consisting of two COP1 proteins and a homo- or hetero-dimer of the SPA proteins and that the interaction with SPA proteins enhances the activity of COP1 towards its targets (Saijo et al., 2003; Zhu et al., 2008). The COP1/SPA complex interacts physically and genetically with phyA, phyB and the cryptochromes and the protein-protein interaction can negatively regulate the function of COP1/SPA and of the photoreceptors (Boccalandro et al., 2004; Jang et al., 2010; Lian et al., 2011; Liu et al., 2011; Seo et al., 2004; Wang et al., 2001; Yang et al., 2001).

The COP1/SPA complex acts on virtually all aspects of light-regulated plant development by controlling different factors that are involved in the photomorphogenic development throughout the lifecycle. The activity of the COP1/SPA complex is also illustrated in figure I-4. The well-characterised substrates of the COP1/SPA complex at the seedling stage include the bZIP transcription factor LONG HYPOCOTYL 5 (HY5), the MYB transcription factor LONG AFTER FAR-RED LIGHT 1 (LAF1) and the atypical bHLH factor LONG HYPOCOTYL IN FAR-RED 1 (HFR1) (Ballesteros et al., 2001; Duek et al., 2004; Fairchild et al., 2000; Hardtke et al., 2000; Holm et al., 2002; Jang et al., 2005; Osterlund et al., 2000; Saijo et al., 2003; Seo et al., 2003; Yang et al., 2005a,b). HY5 is rapidly up-regulated by light and regulates the expression of genes by binding to several LIGHT RESPONSE ELEMENTS (LREs) in promoters. It activates or represses the expression of the target gene (Lee et al., 2007). HY5 predominantly binds to G-Box elements (CACGTG), but also to other LRE (Lee et al., 2007). A large portion of light-regulated genes, around 20% of the genes of the Arabidopsis genome, was shown to be regulated by COP1 and HY5 antagonistically regulates a subset of the COP1 regulated genes (Tepperman et al., 2001; Ma et al., 2001 and 2002). A high number of transcription factors were identified to be expressed under the direct control of HY5 (Lee et al., 2007). In the regulation of adult leaf size, COP1/SPA is proposed to act via the regulation of B-BOX DOMAIN transcription factor BBX21 and HY5 (Fackendahl, PhD Thesis, 2011).The COP1/SPA complex inhibits flowering in short day (SD) conditions by regulating the protein levels of the B-BOX transcription factor CONSTANS (CO) that induces the expression of FLOWERING LOCUS T (FT) and *TWIN SISTER OF FT* (*TSF*) in long day (LD) conditions, two floral inducers that promote the transition from vegetative to reproductive growth (Jang et al., 2008; Laubinger et al., 2006; Liu et al., 2008). It is expected that novel COP1/SPA targets are yet to be discovered as only a subset of the phenotypes of the *cop1* and multiple *spa* mutant can currently be explained with the known targets (Fackendahl, PhD Thesis, 2011; Maier, PhD Thesis, 2011; Falke, Master Thesis, 2009).

The response of the hypocotyl to low R:FR conditions requires the *COP1* gene, as *cop1* mutants display diminished elongation responses compared with the WT (McNellis et al., 1994). *COP1* is required for normal PIF level accumulation (Leivar et al., 2008) and genetically interacts with transcription factors of the BBX family that are involved in the shade avoidance response of the hypocotyl (Crocco et al., 2010). The *bbx* mutants *bbx19, bbx 21* and *bbx 22* exhibit increased elongation responses of hypocotyls to low R:FR treatments compared to the WT, while *bbx18* and *bbx24* display the opposite effect on the hypocotyl length (Crocco et al., 2010). The low R:FR induced hypocotyl elongation of the *cop1-4* mutant is restored when *bbx21* and *bbx22* are introduced into the mutant background, indicating that BBX proteins may act downstream of COP1 and that COP1 may in part exert its function in shade by repression of negative factors (Crocco et al., 2010). Whether *SPA* genes are also involved in the regulation of the SAS, is currently unknown.

I.3 Light signalling in the shade avoidance responses

The shade avoidance responses are tightly regulated by a number of promoting and repressing factors. The elongation responses are negatively regulated by atypical (b)HLH factors in order to prevent overstimulation (Hornitschek et al., 2009; Roig-Villanova et al., 2007; Sessa et al., 2005). These factors include HFR1, which lacks a functional basic domain essential for the binding to DNA (Hornitschek et al., 2009; Sessa et al., 2005). It physically interacts with PIF4 and PIF5 via the HLH domain and forms non-functional heterodimers (Heim et al., 2003; Hornitschek et al., 2009). This repression of elongation promoting factors is in agreement with the observed exaggerated *hfr1* hypocotyl elongation exhibited in response to low R:FR compared with the WT (Sessa et al., 2005; Hornitschek et al., 2009). Overexpression of *HFR1* and especially of truncated versions of the HFR1 protein lead to a diminished hypocotyl elongation in response to simulated shade, which indicates that HFR1 protein levels are negatively regulated in low R:FR conditions to prevent over-accumulation of the negative factor (Galstyan et al., 2011). The transcript levels of *HFR1* are elevated shortly after the onset of low R:FR conditions and they remain elevated for days in prolonged shade conditions providing a negative feedback-loop (Devlin 2003; Sessa et al., 2005). Similarly to *HFR1*, two genes coding for small and atypical bHLH transcription factors, *PHYTOCHROME RAPID REGULATED 1* and *2* (*PAR1, PAR2*), are involved in the repression of the elongation responses to low R:FR. They are swiftly upregulated in response to low R:FR and the induction of the expression can be reversed by high R:FR treatment (Roig-Villanova et al.,

2006). The simultaneous reduction of PAR1 and PAR2 protein levels caused a stronger hypocotyl elongation response to low R:FR conditions compared with the WT, while overexpression of both factors diminished the hypocotyl response (Roig-Villanova et al., 2007). PAR1 (and presumably PAR2 as well) acts on PIF function similar to HFR1 by forming heterodimers that are incapable of binding to the PIF target sequences of promoters (Galstyan et al., 2011; Hao et al., 2012). Additionally, *PIF3-LIKE 1* (*PIL1*) is also highly up-regulated in low R:FR conditions in a phytochrome-dependent manner by the binding of PIF transcription factors to the promoter (Devlin et al., 2003; Salter et al., 2003; Roig-Villanova et al., 2006; Lorrain et al., 2008). In contrast to *HFR1* and *PAR1/2*, positive and negative functions in the shade avoidance response have been assigned to *PIL1* in different studies, suggesting a more complex function (Salter et al., 2003; Roig-Villanova et al., 2007).

Two positive regulators of the shade avoidance responses are the homeodomain-leucine zipper transcription factor, ARABIDOPSIS THALIANA HOMEOBOX 2 (ATHB2) and ATHB4, which are implicated in the light-hormone interaction necessary for full shade response (Carabelli et al., 1993 and 1996; Roig-Villanova et al., 2006; Sorin et al., 2009). Overexpressors of *ATHB2* exhibit longer hypocotyls, while reduced levels lead to shorter hypocotyls compared with the WT (Steindler et al., 1999). ATHB2 is also strongly up-regulated in low R:FR conditions by the PIFs (Lorrain et al., 2008). Furthermore, the regulation of *ATHB2* in the first hour of the low R:FR-dependent response is regulated by *COP1* in an *HY5*-independent manner (Roig-Villanova et al., 2006). Factors that promote elongation responses to low R:FR also include extracellular proteins that modify the cell wall (Cosgrove et al., 2005). Two main classes of cell wall modifying enzymes are involved in cell wall loosening. First, the expansins form a large family of nonenzymatic proteins in the cell wall that rapidly promote cell wall extension in a pH-dependent manner (Cosgrove et al., 2000). The expansins function equally in white light and shade and are solely influenced by acidification of the cell wall (Cosgrove et al., 2005). Second, the XYLOGLUCAN ENDOTRANSGLUCOSYLASE / HYDROLASES / XYLOGLUCAN ENDOTRANS-GLUCOSYLASE/HYDROLASES – RELATED PROTEINS (XTH/XTR) are a large family of enzymes that modify the xyloglucans that crosslink the cellulose fibres of the cell wall (Eklöf et al., 2010).

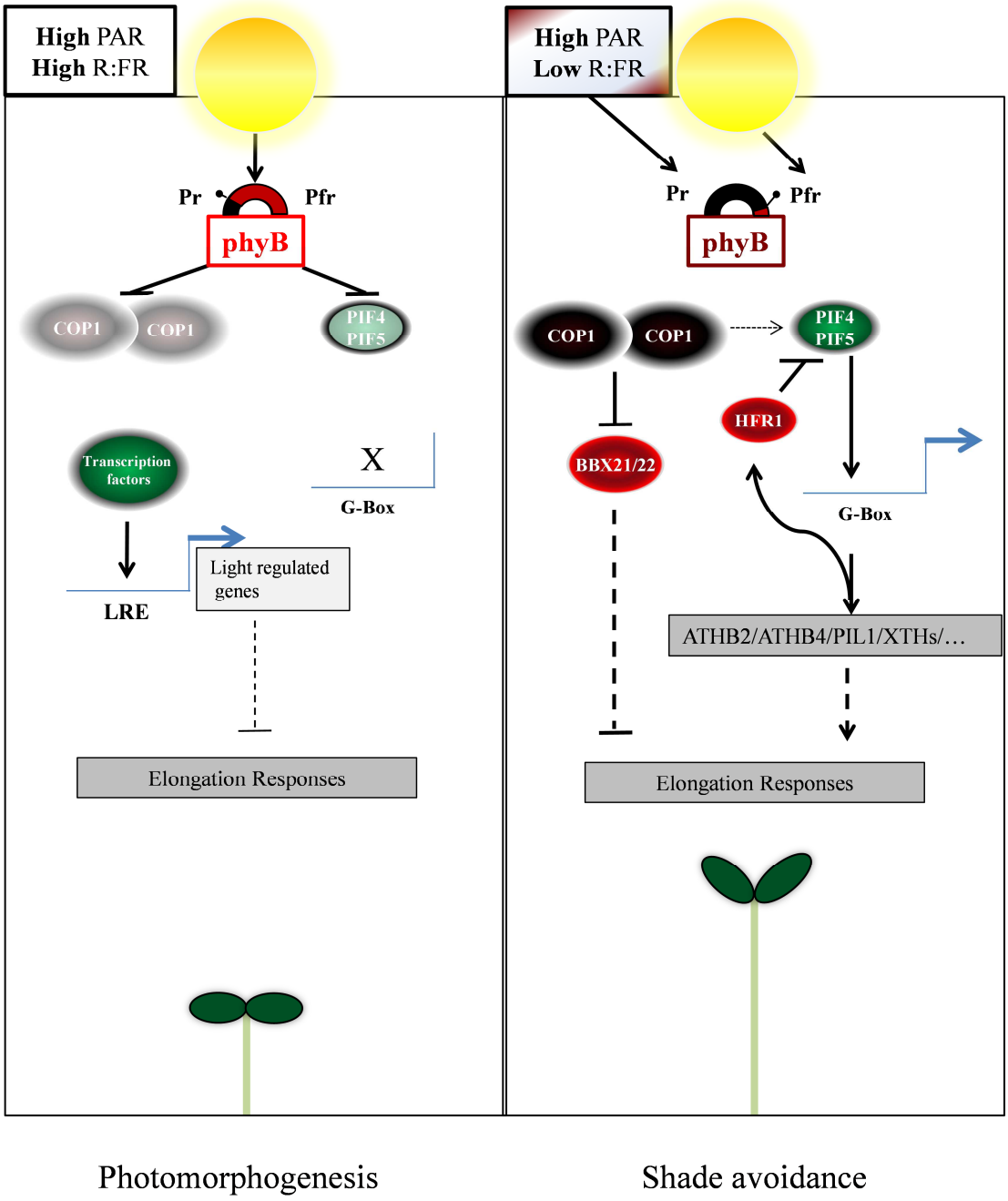

Figure I-5: Molecular network of the shade avoidance response in *Arabidopsis thaliana*. In open sunlight, the COP1 and the PIFs are inactivated by the active Pfr form of phyB and elongation responses are inhibited. In low R:FR, the photoequilibrium of phyB is shifted to the inactive Pr form and COP1 and the PIFs are activated and promote the shade avoidance elongation responses. COP1 acts on BBX21/21 that inhibit elongation and the PIFs promote the expression of elongation promoting factors. HFR1 represents a feed-back loop that negatively acts on the PIFs.

They are required for the loosening of the wall prior to cell elongation (Cosgrove et al., 2005). In contrast to the expansins, XTH function is specifically enhanced in petioles in response to shade conditions, with a distinct set of XTH enzymes operating preferentially in different shade conditions (Sasidharan et al., 2010). Importantly, a *xtr7/xth15* knock-out line shows no induction of growth rate of the petioles in low R:FR conditions and *XTR7* is an established target gene of PIF4 and PIF5 that is up-regulated in response to low R:FR conditions (De

Lucas et al., 2008; Lorrain et al., 2008; Sasidharan et al., 2010). The factors that regulate elongation responses in low R:FR conditions are summerised in figure I-5.

The control of accelerated flowering in response to low R:FR conditions is primarily regulated by phyB and to a minor extend by phyD (Devlin et al., 1999; Halliday et al., 1994). In low R:FR conditions, phyB has been proposed to act independently of the CO pathways on FT expression via PHYTOCHROME AND FLOWERING 1 (PFT1) transcription factor (Cerdán and Chory, 2003). Before flowering, even in short days, a large increase in *FT* transcript levels in *phyB* mutant compared with WT has been reported. However, the acceleration of flowering has also been observed to require *GIGANTEA* (GI) and *CO* and the role of *PFT1* has been questioned (Kim et al., 2008: Wollenberg et al., 2008). The early flowering in response to close competitors can be suppressed by high FLOWERING LOCUS C (FLC) levels in a dose-dependent manner, which causes later flowering in shade in some Arabidopsis accessions (Adams et al., 2009; Wollenberg et al., 2008). Loss of phytochrome function overrides any FLC effect on FT expression, as in a *phyB phyD phyE* triple mutant, high *FLC* level cannot inhibit *FT* induction (Wollenberg et al., 2008).

Taken together, the SAS mainly consist of elongation processes and the acceleration of flowering. Both depend largely on phyB inactivation and downstream signalling events that involve promoting and inhibiting factors. COP1 is a central regulator of light-dependent plant development downstream of several photoreceptors including phyB and has been assigned an important function in the hypocotyl elongation process in response to low R:FR. Whether the COP1-interacting SPA proteins also contribute to the control of the shade avoidance responses remains an open question. Preliminary results obtained under my supervision point towards a function for *SPA* genes in the shade avoidance response of seedlings (Stephen Dickopf, Master Thesis, 2011; Jan Sahm, Examensarbeit, 2010).

I.4 Interactions of the light and auxin pathways

Auxin is a phytohormone that is regarded as the master regulator of plant development (Jaillais and Chory, 2010). Auxin acts on cell elongation, proliferation and differentiation and is required for proper embryogenesis, root initiation, vascular patterning and apical dominance. Furthermore, it drives directional plant growth reactions, such as phototropism and gravitropism, towards or away from environmental cues, highlighting the interplay between sensory input and the mediation of plant growth responses by auxin. The levels of active auxin are tightly controlled by biosynthesis, conjugation and breakdown (Tam et al.,

2000; Staswick et al., 2002; Staswick, 2005). Furthermore, auxin is actively transported by cellular import and export and the responsiveness of the target tissues can be modulated by differential regulation of the auxin signalling machinery (Fig. 1-6). Light signalling can be found to manipulate virtually all levels of the auxin pathway (Halliday et al., 2009).

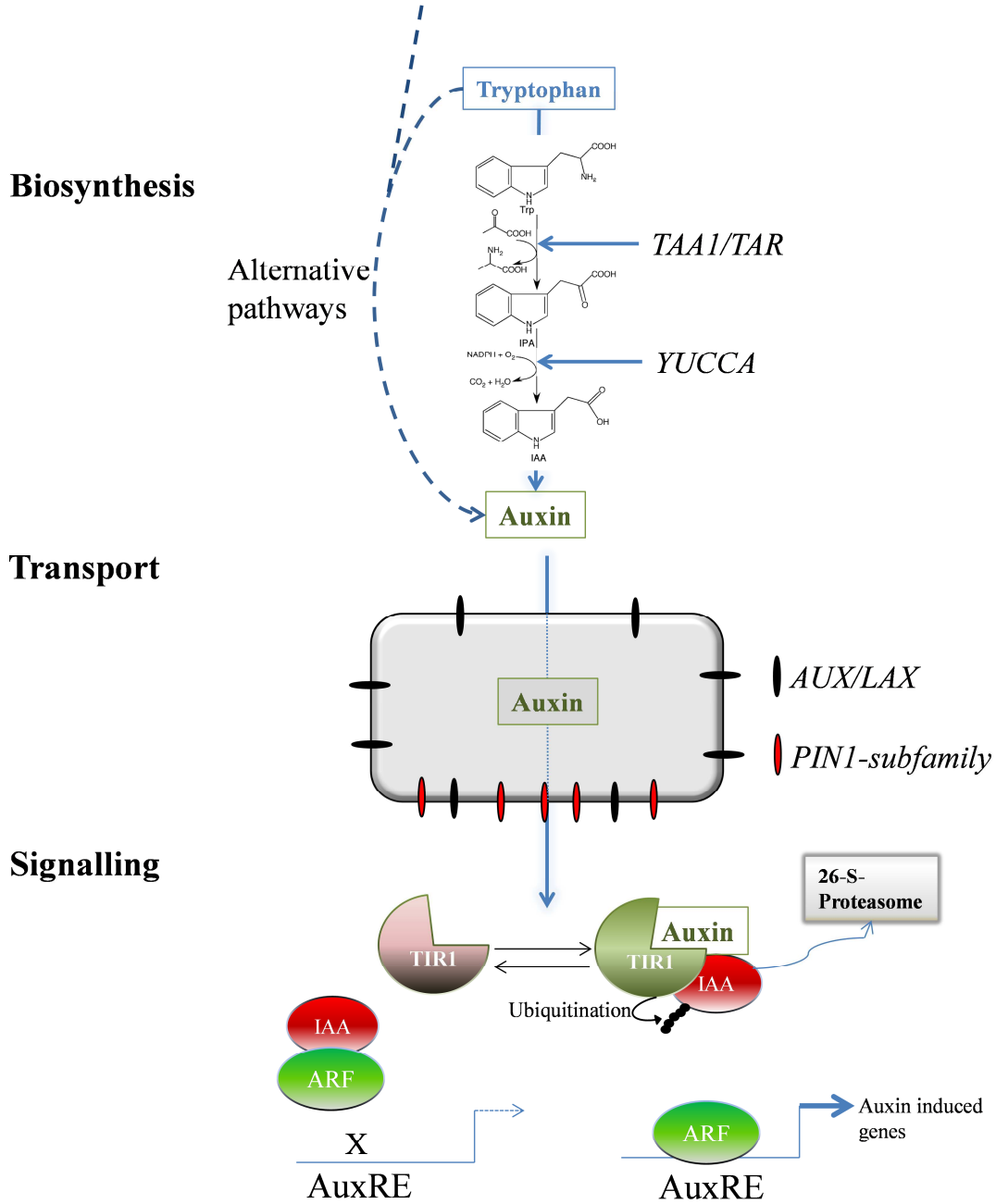

Figure I-6: The pathway of auxin biosynthesis, transport and signalling. IAA is mainly synthesized by TAA1 and YUCCA from tryptophan and transported from the site of biosynthesis via auxin influx and efflux carriers of the AUX/LAX and PIN families. Auxin can be perceived by the TIR1-auxin receptor that targets Aux/IAA protein for degradation that negatively regulated ARF transcription factors. ARFs then bind Auxin response elements (AuxRE) to induce auxin regulated genes.

I.4.1 Auxin-Biosynthesis, conjunction and catabolism

Indole-3-acetic acid (IAA) is the main active auxin in Arabidopsis and mainly synthesized from tryptophan (Tao et al., 2008). The *TRYPTOPHAN AMINOTRANSFERASE 1* (*TAA1)* is a key IAA biosynthesis gene that has been isolated from independent screens for altered auxin associated phenotypes, which include hypocotyl elongation in simulated canopy shade (*SHADE AVOIDANCE 3* (*SAV3*), ethylene dependent root elongation (*WEAK ETHYLEN INSENSITIVE 8* (*WEI8*)) and the resistance to auxin transport inhibitor treatment (*TRANSPORT INHIBITOR RESPONSE 2* (*TIR2*)) (Stepanova et al., 2008; Tao et al., 2008; Yamada et al., 2009). TAA1 possesses enzymatic activity, converting tryptophan to indole-3-pyruvate (IPA) which is subsequently metabolised to IAA (Tao et al., 2008; Stepanova et al., 2008; Mashiguchi et al., 2011). Four *TAA-RELATED* genes *TAR1-TAR4* are present in the Arabidopsis genome, with *TAR1* and *TAR2* being the most related family members to *TAA1*, exhibiting additive functions in IAA biosynthesis (Stepanova et al., 2008). Single and multiple mutations in the genes coding for the TAA1/TAR protein family result in severe auxin related phenotypes, including loss of response to gravity, deficit in the formation of primary roots and reduced length or complete absence of hypocotyls (Stepanova et al., 2008). TAA1 is the predominant enzyme of the TAA1/TAR family involved in the rapid increase of free auxin levels in response to shade (Tao et al., 2008). A second family of enzymes involved in auxin biosynthesis, the flavin monooxygenase-like YUCCA (YUC) proteins, consists of 11 members in Arabidopsis with largely overlapping functions (Cheng et al., 2006; Cheng et al., 2007; Zhao et al., 2001). *YUC* genes are equally important as the *TAA1* gene family with regard to the development of Arabidopsis plants. Due to redundancy in the gene family, *YUC* genes have first been noticed by high-auxin phenotypes resulting from the overexpression of single *YUC* (Zhao et al., 2001). Multiple *yuc* mutants exhibit defects as early as during embryogenesis, because auxin produced by *YUCCA* gene is essential for proper development of the embryo (Cheng et al., 2007). Seedling development, the development of the vascular tissue and also the development of flowers depend in the same way on *YUC* function (Cheng et al., 2006, Cheng et al., 2007). Despite the long-lasting proposition of multiple parallel auxin biosynthesis pathways, recent findings favour a major straightforward two-step auxin biosynthesis pathway comprising of the TAA1/TAR family of enzymes and the YUC enzymes, in which TAA1 produces IPA from tryptophan and YUC metabolises IPA to IAA (Mashigushi et al., 2011; Phillips et al., 2011; Stepanova et al., 2011; Won et al., 2011). IAA is oxidized to an inactive form by an unknown mechanism and can also be temporally inactivated by conjugation to a sugar, amino acid or methyl-group and may subsequently be

reactivated or degraded (Li et al., 2007; Ljung et al., 2002; Woodward and Bartel, 2005; Yang et al., 2008). IAA is mainly produced in the cotyledons, young leaves and the meristems and transported to the other parts of the plant, but further local auxin biosynthesis exists in the meristem of the root, the tips of lateral roots and presumably further locations (Ljung et al., 2001)

I.4.2 Auxin transport

Auxin is transported from the sites of synthesis over long distances throughout the plant. First, IAA can be loaded to the phloem and passively distributed via the stream to all sink tissues. Second, besides this passive and fast auxin flow, an active auxin transport mechanism exists, the polar auxin transport (PAT) (Gao et al., 2002). This mode of transport is considered to be unique among the phytohormones and is dependent on a set of auxin carriers that function in each individual cell contributing to the PAT (Delbarre et al., 1996). Auxin enters the cell by diffusion and active uptake that is mediated by 11-transmembrane AUXIN RESISTANT1 / AUXIN RESISTANT1-LIKE (AUX/LAX) carriers (Bennett et al., 1996; Swarup et al., 2004) (see Fig 1-5). In the cytoplasm, auxin is largely deprotonated due to the higher pH and the charged auxin is unable to diffuse out of the cell. PIN FORMED (PIN) proteins are essential components of the major efflux carriers of auxin from the cytoplasm to the apoplast (Friml et al., 2002). They form a family of eight members with two subgroups. The PIN1 subgroup members (PIN1, PIN2, PIN3, PIN4, and PIN7) can be dynamically relocated to the apical or basal sides of the plasma membrane (PM) by phosphorylation or dephosphorylation, respectively, which influences the direction of auxin efflux (Friml et al., 2002, 2004; Sukumar et al., 2009; Zhang et al., 2009). The phosphorylation status of the PIN proteins is regulated by PINOID (PID) serine-threonine kinases and the PROTEIN PHOSPHATASE 2A (PP2A) (Christensen et al., 2000; Friml et al., 2004; Sukumar et al., 2009). Furthermore, three ATP-binding cassette class B (ABCB) transporters are specific for auxin transport and efficiently exclude it from the cytoplasm to the apoplast (Kubeš et al., 2012; Wu et al., 2010).

I.4.3 Auxin signalling

Two types of auxin receptors were identified to date. IAA is bound by the AUXIN BINDING PROTEIN 1 (ABP1) auxin receptor that contributes to the early phase of auxin induced elongation independent of regulation of gene expression (Perrot-Rechenmann, 2010). Auxin perception by ABP1 was shown to promote the activity of two Rho GTPases, which act on the lobbing of pavement cells (Xu et al., 2010). A family of six F-Box E3-ubiquitin ligases named

TRANSPORT INHIBITOR RESPONSE1 (TIR1)/ AUXIN SIGNALLING F-BOX (AFB) also possess auxin receptor function (Gray et al., 2001; Dharmasiri et al., 2005; Kepinski and Leyser 2005). Upon auxin binding to the catalytic site of the TIR1/AFB receptors, the E3-ubiquitin-ligase binding and activity towards AUXIN INSENSITIV/ INDOLE-3-ACETIC ACID (Aux/IAA) proteins are strengthened and they become ubiquitinated and subsequently degraded by the 26S-proteasome (Kepinski and Leyser, 2004; Tan et al., 2007). The Aux/IAA proteins are inhibitors of AUXIN RESPONSE FACTORS (ARF), as they hetero-dimerization via conserved domains and prevent their binding to promoters of auxin-induced genes (Overvoorde et al., 2005). Hence, auxin induces the transcription of target genes by activation of the ARF proteins (see fig. I-5). ARF transcription factors form a family of 23 members in Arabidopsis and bind to AUXIN RESPONSE ELEMENTS (AuxREs) in promoters of auxin-responsive genes (Okushima et al., 2005; Ulmasov et al., 1997 a). The majority of the 29 *Aux/IAA* genes in Arabidopsis are rapidly auxin-induced, which provides a primary negative feed-back loop on auxin signalling, shaping the auxin signal (Abel et al., 1994; Remington et al., 2004).

I.4.4 Interactions of light signalling and the auxin response

Like other light signalling mutants, multiple *spa* mutants and *cop1* mutants exhibit short hypocotyls and dwarfed plant growth with the number and the size of leaf cells being diminished (Fackendahl, Phd thesis, 2011; Ranjan et al., 2011; Laubinger et al., 2004; Fittinghoff et al., 2006). It remains an open question, whether these phenotypes are caused by misregulation of the auxin response (Ranjan et al., 2011). Non-cell-autonomous functions for *SPA* genes have been described for seedling growth, the induction of flowering time and the regulation of leaf size by the tissue-specific expression of SPA1 protein in *spa* triple mutants (Ranjan et al., 2011). Light can act on all levels of the auxin pathway. Auxin biosynthesis was shown to be regulated negatively by phyB, which acts by lowering TAA1-dependend auxin production (Tao et al., 2008). The PAT is also controlled by light. The intracellular distribution of PIN proteins is controlled by blue light via HY5 action in root cells (Laxmi et al., 2008). The ABCB19 auxin transporter is under the control of cry1 and phyB, which also influences auxin flow in a light-dependent fashion (Wu et al., 2010). Additionally, the root to shoot ratio of auxin is controlled by phyB and cry1. Furthermore, *phyA phyB* double mutants are largely deficient in the shoot to root distribution of auxin, causing aberrant growth responses of the root (Salisbury et al., 2007). Furthermore, the auxin signalling network is regulated by light signalling. Several *iaa* gain-of-function mutants exhibit growth defects in

light-associated phenotypes, such as in the *HY2/IAA3* gene, which regulates auxin and light-mediated development of the shoot and the root (Tian and Reed, 1999; Soh et al., 1999). Strinkingly, a high number of auxin-related genes is among the genes rapidly regulated by R and FR light (Tepperman et al., 2001 and 2006; Ma et al., 2001; Devlin et al., 2003). It has been noticed that several *Aux/IAA* genes are light-regulated (Tepperman et al., 2001 and 2006). Studies of an *IAA19 promoter::GUS* fusion revealed that *IAA19* expression was strongly repressed in a time and fluence-dependent manner in white light (Saito et al., 2007). *IAA19* is strongly auxin-induced, mainly in the hypocotyl and the root, dependent on ARF7 that binds to the *IAA19* promoter (Tatematsu et al., 2004). Due to the strong up-regulation by auxin, the *IAA19* promoter has been used to visualize auxin signalling (Keuskamp et al., 2010). Interestingly, the transcript levels of *IAA29* have been reported to be down-regulated in a multiple *pif* mutant and up-regulated in a PIF4/PIF5 dependent manner at the end of the night in SD grown plants (Leivar et al., 2009; Kunihiro et al, 2011). Whether PIF4 and PIF5 directly bind to the *IAA29* promoter to modulate auxin signalling or whether up-regulation of *IAA29* requires additional factors has not been solved. A direct regulation of Aux/IAA genes by light signalling has also been proposed due to the HY5 binding to the promoters of the R repressed *IAA8*, *IAA16*, *IAA17* and *IAA18* (Lee et al., 2007). Furthermore, the transcript abundance of *IAA7* and *IAA14* are lower in *hy5* mutants compared to the WT and HY5 can bind the *IAA7* promoter *in vitro* (Cluis et al., 2004). The majority of *SMALL AUXIN UP RNA* (*SAUR*) genes are rapidly and strongly auxin-induced and they code for small proteins unique to plants that have repeatedly been noticed to positively correlate with cell elongation (Knauss et al., 2003; Esmon et al., 2006). The *SAUR19-24* subfamily promotes cell expansion in an auxin-dependent fashion and the overexpression of SAUR19 in the *pif4* mutant restores the auxin-induced hypocotyl elongation response to high temperatures (Franklin et al., 2011; Spartz et al., 2012). SAUR proteins may in part act on auxin transport by association to the PM (Spartz et al., 2012). Several *SAUR* promoters have also been identified as direct targets of HY5-binding (Lee et al., 2007).

Two systems are employed to monitor the auxin response. A synthetic auxin-responsive promoter sequence has been generated that contains tandem repeats of AuxRE core motifs DR5 promoter that is fused to reporter genes (e.g. GUS and LUC).The DII-domain of IAA28 was fused to VENUS (fast maturating YELLOW FLUORESCENT PROTEIN (YFP)), which was shown to represent a more direct and more sensitive auxin signalling sensor system (Ulmasov et al., 1997; Brunoud et al., 2011).

Due to the complexity of the auxin pathway, light repression of auxin-up-regulated genes could be caused by a direct or indirect regulation via the auxin signalling pathway (Tepperman et al., 2006). The characteristic promoter sequences of auxin-regulated genes are auxin response elements (AuxRE) and most of them share the common consensus core motif TGTCT(C), though cryptical AuxRE exist that do not share the consensus (Ulmasov et al., 1997 b; Walcher and Nemhauser, 2011). Light regulated genes carry LRE in their promoter sequences, such as G-BOX motifs that can be bound by PIF and HY5 and may promote or repress the expression of the gene (Martínez-García et al., 2000; Lee et al., 2007).

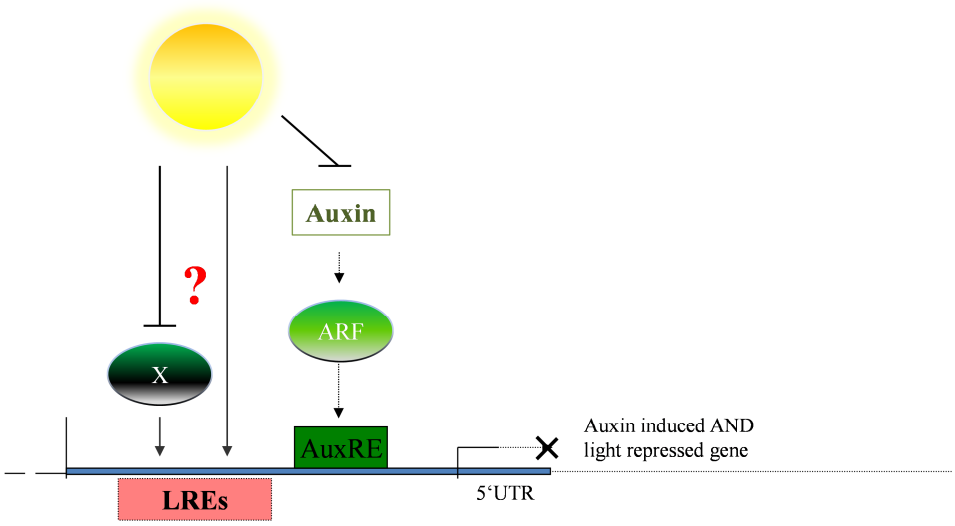

Figure I-7: Possible modes of the regulation of light- and auxin-regulated genes. Shown is a representation of the pathways that could influence the regulation of auxin-up-regulated and light-repressed genes. This applies to a large subset of auxin-associated genes.

For the two *Aux/IAA* genes, *IAA19* and *IAA6*, three AuxRE motifs were identified by sequence analysis within the 300-bp promoter region (Remington et al., 2004). A G-Box motif has also been detected in the 1000-bp promoter of *IAA19* (Sibout et al., 2006).

For virtually all auxin-induced genes, it remains to be solved, if direct light-regulation is involved in their light-repression or if their regulation is only controlled by AuxRE. Therefore, in order to unravel the input of light to the auxin signalling pathway, it will be important to investigate the interaction of light and auxin on the level of individual promoters to dissect direct and indirect regulation of gene expression (Fig. I-7).

By controlling the regulation of auxin-regulated genes, such as *IAA19*, light could directly influence auxin-responsiveness of cells and tissues to modulate the output of the auxin system, such as elongation responses (Cluis et al., 2004; Sibout et al., 2006; Tepperman et al., 2006).

I.4.5 Light regulation of the auxin response in the SAS

The interplay of light and auxin responses is also beginning to be unravelled in the SAS. The shade induced elongation responses of the hypocotyl require the modulation of auxin biosynthesis, transport and signalling. TAA1 is essential for the increase of auxin levels in low R:FR conditions which is in turn required for the elongation response of the seedling (Tao et al., 2008). The gene expression of auxin-responsive genes (e.g. *IAA* genes) is elevated in low R:FR conditions and the auxin response in cotyledons is higher in low R:FR than in high R:FR (Devlin et al., 2003; Tao et al., 2008). However, the transcript level of *TAA1* is not responsive to the shade treatment (Tao et al., 2008). The two family members *YUC1* and *YUC4* are expressed in the aerial part of the seedling and they are together important for the hypocotyl elongation in low R:FR compared to high R:FR (Won et al., 2011). Furthermore, overexpression of *YUC1* in the *sav3* mutant background restores the shade avoidance phenotype of the mutant. *YUC2*, *YUC5*, *YUC8* and *YUC9* transcript levels are elevated in shade, but *yuc* multiple mutants including *yuc8 yuc9* and the *yuc3 yuc5 yuc7 yuc8 yuc9* quintuple mutant (*yuc-Q*) exhibit normal elongation response of the hypocotyl to simulated shade (Tao et al., 2008). This indicates that the YUC enzymes that catalyse the rate-limiting step in the auxin biosynthesis may be up-regulated in shade conditions to increase auxin levels (Tao et al., 2008; Won et al., 2011). The transport of auxin is also essential for the low R:FR dependent elongation response of the hypocotyl (Steindler et al., 1999; Pierik et al., 2009; Keuskamp et al., 2010). PIN3 is required for the hypocotyl elongation response, up-regulated on the transcript level and the stabilized on the protein level in shade avoidance conditions (Devlin et al., 2003; Friml et al., 2002; Keuskamp et al., 2010). Furthermore, PIN3 is relocated in order to redirect auxin flow towards the epidermis, which promotes auxin-responsive gene expression in the epidermis (Friml et al., 2002; Keuskamp et al., 2010). Also, *SAUR* genes, which function in auxin regulated elongation processes, are up-regulated in shade conditions (Roig-Villanova et al., 2007).

I.5 Aims of this thesis

(I) **Investigation of functions of the *SPA* genes and *COP1* in shade avoidance responses:** *COP1* is an important positive regulator of the shade avoidance syndrome in seedlings, but a function for *SPA* genes in shade avoidance has not been described. Thus, the first aim of this study was to unravel a role of *SPA* genes in shade avoidance including an investigation of functions of *COP1* and the *SPA* genes in shade avoidance responses of adult plants. The examination was carried out on the phenotypic and the molecular level.

(II) **Analysis of auxin response in *spa* mutant backgrounds:** The *spa* mutants exhibit seedling and adult leaf phenotypes that are phenocopied by auxin signalling and auxin biosynthesis mutants. Furthermore, SPA1 was implicated in non-cell-autonomous signalling. Thus, the second aim of this study was to investigate the auxin response in *spa* mutants. *DR5::GUS* was introduced in the *spa1 spa2 spa4* background and analysed alongside the *spa1 spa3 spa4 DR5::GUS* in darkness, light, SAS and adult plant development.

(III) **Dissection of the light- and auxin-regulation of auxin-induced and light-repressed genes:** The evidence for light-regulation of auxin-induced genes is substantial, but whether direct light signalling to the promoters of auxin-induced genes down-regulates them in the light is still unexplored. So, the third aim of this study was to investigate the regulation of genes that are auxin-induced and light-repressed on the level of the promoters. To this end, *promoter::luciferase* constructs were generated and analysed for the regulation in darkness vs. red light (Rc).

II. Results

II.1 *SPA* gene function in the SAS

Several *cop1* mutants exhibit a reduced elongation response to simulated shade and the *cop1* mutation genetically interacts with two *bbx* mutations in shade avoidance (Crocco et al., 2010; McNellis et al., 1994). As SPA proteins commonly act together with COP1 (reviewed in Hoecker, 2005), functions of *SPA* genes in shade avoidance were tested.

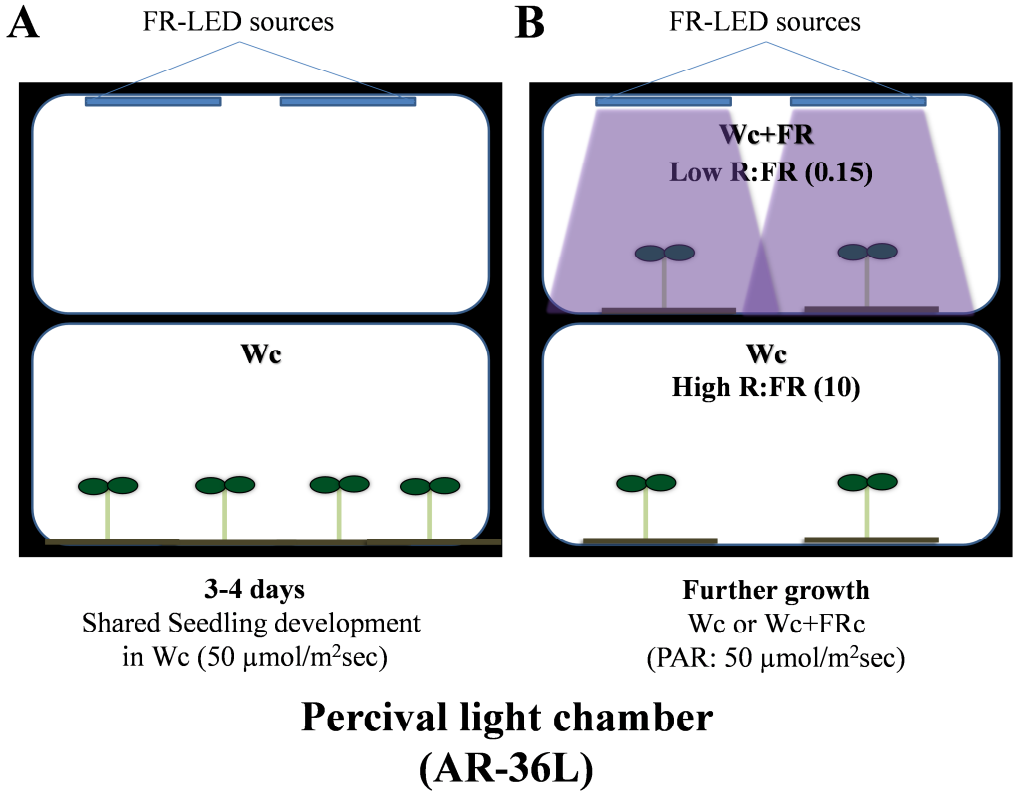

Figure II-1: Simulated shade set-up for adult plant analysis. All seedlings were initially grown in continuous white light (Wc) in the lower shelf (**A**) and a subset subsequently shifted to Wc supplemented with continuous far-red light from LED light sources (Wc+FRc) in the upper shelf (**B**). The Wc+FRc set-up resulted in a lower R:FR ratio of the ambient light than in Wc alone, but the PAR was identical in both light conditions. Experiments with seedlings were analysed in two identical light chambers employing the same strategy.

The simulated shade conditions employed in this study consisted of continuous white light (Wc) supplemented with additional continuous FR light (Wc+FRc), resulting in a lower R:FR ratio in comparison with the Wc light condition alone, but an unchanged PAR. Only de-etiolated seedlings are capable of exhibiting longer hypocotyls in response to low R:FR conditions compared with sunlight conditions. Dark-grown seedlings react to low R:FR light treatment with inhibition of the hypocotyl elongation, due to the high activity of dark-

accumulated phyA (Smith et al., 1997; Strasser 2010). Therefore, all seedlings were grown for three to four days in Wc, which was provided by fluorescent light tubes (chamber for adult plant growth) or white light LED light sources (chambers for seedling experiments). Subsequently, one part of the seedlings was moved to the low R:FR conditions that were generated by additional FR LED light sources (see Fig. IV-1 for spectral analyses). The growth strategy for the analysis of adult plant responses to simulated shade is exemplarily presented in figure II-1.

First, soil-grown WT seedlings were analysed in the newly constructed simulated shade conditions and displayed elongated hypocotyls in low R:FR conditions compared to Wc (data not shown; Sahm, 2010). Additionally, seedlings were grown on MS plates in the simulated shade in order to allow transcript level analyses and other applications. When the response of the hypocotyl to low R:FR was analysed in seedlings grown on MS plates, no elongation response to the low R:FR treatment was observed compared with the seedlings grown in Wc. It was reasoned that the shade avoidance response might depend on the dark surface of the soil or other properties that differ between soil and MS plates in our set up. The hypocotyl elongation to low R:FR conditions could be restored in seedlings grown on blackened MS medium (agar supplied with 1% activated charcoal, black MS), but not on MS plates, which had a blackened bottom (supplemental figure S1). This suggests that the obscureness of the soil was the decisive factor for the elongation response of the seedlings to low R:FR in contrast to the translucent MS medium. Thus, all shade experiments were either performed on soil where indicated or on MS medium that contained 1% charcoal (black MS).

II.1.1 Phenotypic and molecular analysis of *spa* mutants and the *cop1* mutant in low R:FR conditions

II.1.1.1 SPA genes are essential for the shade avoidance responses of seedlings

In order to investigate functions for *SPA* genes in the SAS, *spa* single and multiple mutants were analysed in the Wc and Wc+FRc conditions. Seedlings were grown in Wc for three days and shifted to low R:FR conditions (Wc+FRc) for additional three days, while a second set of seedlings was kept in Wc. WT seedlings responded to the low R:FR treatment with increased hypocotyl elongation, exhibiting an approximately two times longer hypocotyl compared to seedlings grown in Wc (Fig. II-2 A,B). This demonstrates that the low R:FR set-up triggered an elongation response in seedlings. The *phyB-9* mutant displayed long hypocotyls in Wc,

when compared to the WT and shorter hypocotyls in low R:FR conditions when compared to Wc.

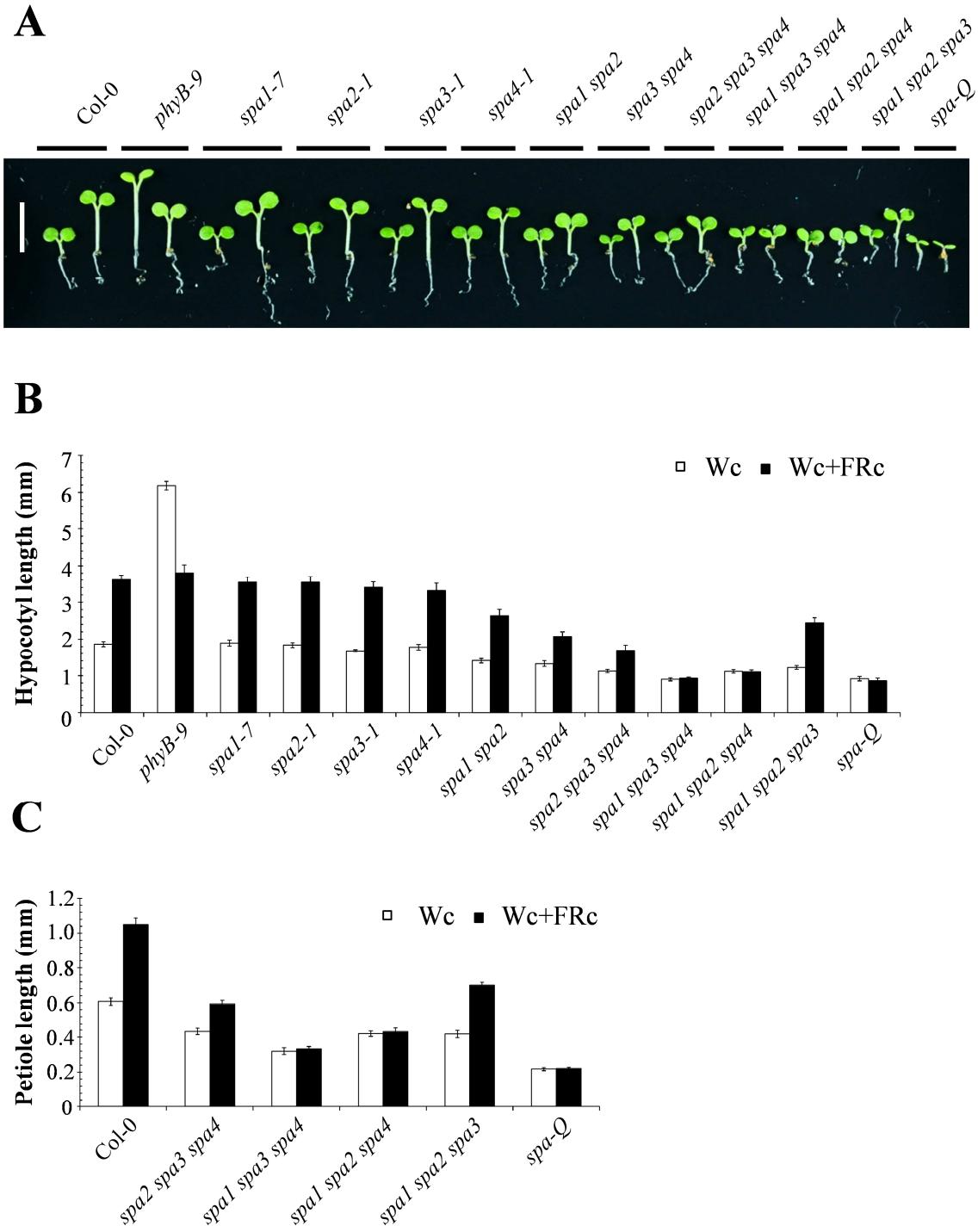

Figure II-2: *SPA* genes are essential for hypocotyl and petiole elongation in low R:FR. A) Shade phenotype of six-day-old black MS grown *spa* mutant seedlings. Within each pair of seedlings of one genotype, seedlings grown in continuous white light (Wc) are on the left, seedlings grown in continuous white light supplemented with far-red light (Wc+FRc) on the right. The white bar represents 5 mm. **B)** Hypocotyl length of *spa* mutants in Wc and Wc+FRc (>15 seedlings were measured, data presented as mean ± standard error of the mean (SEM)). **C)** The petiole length of the cotyledons of triple and quadruple *spa* mutants (the length of the two petioles per seedling was averaged, >15 seedlings were measured, data presented as mean ± SEM)

The reduction of hypocotyl elongation in the *phyB* mutant was described before and was most likely caused by the phyA-mediated HIR triggered by the higher FR fluences (Devlin et al., 2003). Thus, the observed low R:FR triggered elongation responses reflect the net sum of low R:FR dependent increase and FR-HIR dependent decrease of the hypocotyl length of the seedlings (Devlin et al., 2003). The *spa* single mutants did not show an altered elongation response of the hypocotyl compared with the WT seedlings. However, the *spa1 spa2 spa3 spa4* quadruple mutant (*spa*-Q) did not show a hypocotyl elongation response to low R:FR compared with Wc. This indicates that the *SPA* genes are essential for the response of hypocotyls to low R:FR and function redundantly. Both tested double mutants, *spa1 spa2* and *spa3 spa4* exhibited longer hypocotyls in low R:FR than in Wc, but the elongation response to simulated shade was diminished compared with the WT and the single mutants (Fig. II-2 B). Similarly, the *spa2 spa3 spa4* and *spa1 spa2 spa3* triple mutants responded with a significant hypocotyl elongation response to the simulated shade conditions. The hypocotyl elongation response of the *spa1 spa2 spa3* mutant to low R:FR conditions compared to the Wc conditions was stronger than the hypocotyl elongation response of the *spa2 spa3 spa4* mutant that showed a reduced elongation response to low R:FR. The hypocotyls of the two other triple mutants failed to respond to the applied low R:FR treatment, exhibiting the same hypocotyl length under Wc and simulated shade. These results suggest that *SPA* genes have redundant, but also distinct functions in the elongation response of the hypocotyl to low R:FR treatment. *SPA1* and *SPA4* are sufficient to sustain a shade avoidance response of seedlings, when the other three *SPA* genes are mutated.

The petiole elongation of the cotyledons of six-day-old plants in response to Wc and Wc+FRc treatment was also analysed. It was observed that the *spa1 spa3 spa4* and the *spa1 spa2 spa4* triple mutants failed to exhibit a petiole elongation response to the low R:FR conditions compared with the elongation response seen in the WT (Figure II-2 C). The same was true for the *spa*-Q mutant. The two other triple mutants still expressing SPA1 or SPA4 displayed an elongation response of the petioles to the low R:FR treatment. Again, the *spa1 spa2 spa3* mutant showed a stronger response to the low R:FR treatment than the *spa 2 spa3 spa4* mutant. Thus, similar results were obtained for the elongation response of the hypocotyl and the elongation response of the petioles of the cotyledons. This suggests that both responses are connected by a common mechanism which is controlled by the *SPA* genes.

II.1.1.2 Transcript analysis of shade marker genes in *spa* mutants and *cop1-4*

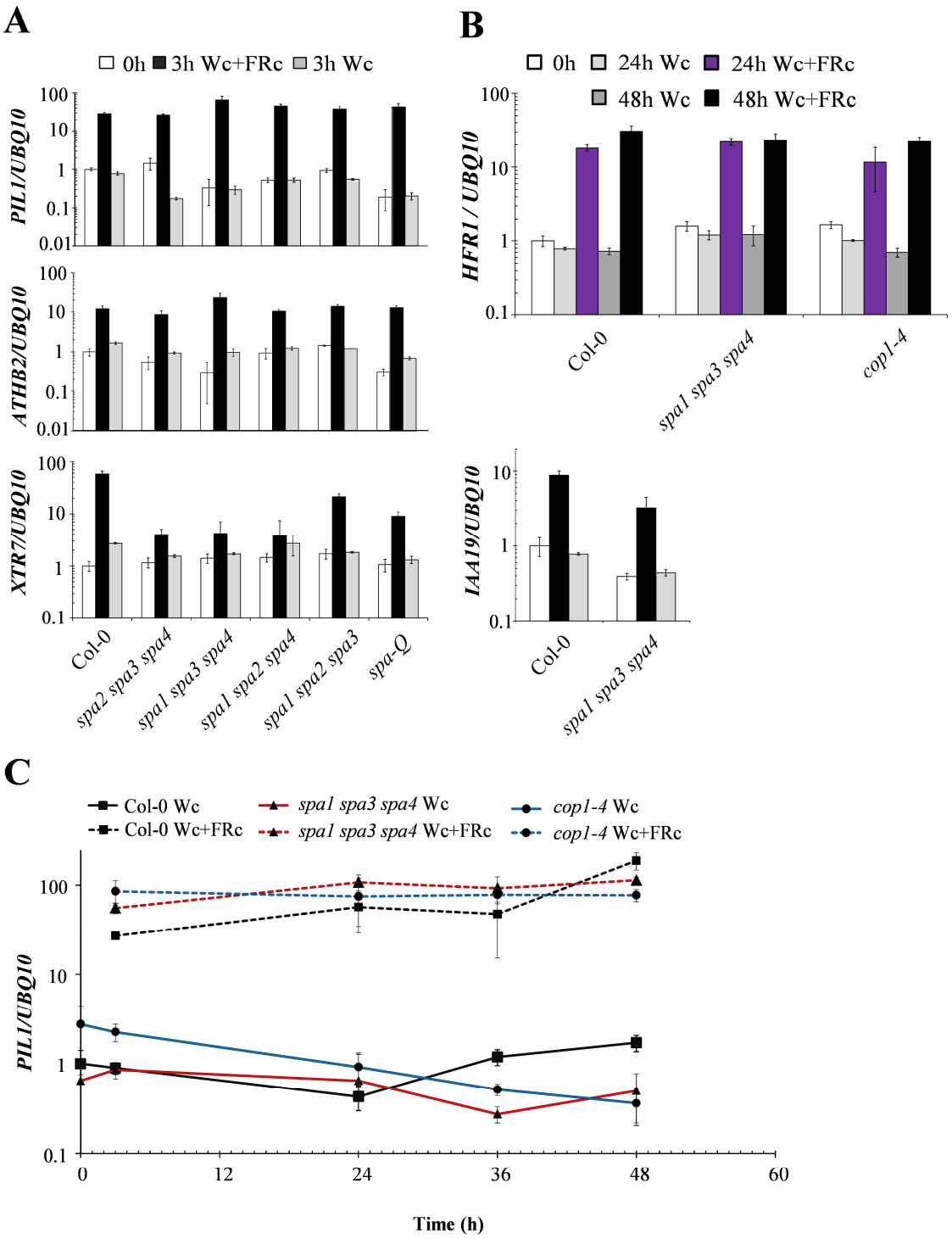

Figure II-3: Expression of early shade marker genes in *spa* multiple mutants. A) Transcript levels of *PIL1*, *ATHB2*, *XTR7/XTH15* and *IAA19* were analysed with quantitative qRT-PCR. Seedlings were grown for 4 d in continuous white light (Wc) and subsequently shifted to low R:FR conditions or kept in Wc for 3 h. *UBQ10* was used as endogenous control. Data represent the mean of three biological replicates ± SEM. **B)** Transcript level of *HFR1* were analysed with qRT-PCR. Seedlings were grown for 4 d in Wc and subsequently shifted to low R:FR conditions or kept in Wc for 24 h and 48 h. *UBQ10* was used as endogenous control. All data was calibrated to 0 h Wc sample and represents the mean of three biological replicates ±SE.**C**) Time-course analysis of the transcript levels of *PIL1* in Wc and low R:FR conditions. *UBQ10* was used as endogenous control. All data was calibrated to 0 h Wc sample and represents the mean of three biological replicates ± SEM.

Genes, which are swiftly up-regulated by shade conditions, are referred to as e*arly shade marker genes*. These include the transcription factors *ATHB-2*, *PIL1* and *HFR1* (Lorrain et al., 2008) and genes encoding enzymes that are involved in cell wall modification, such as *XTR7/XTH15* (De Lucas et al., 2008). Furthermore, a set of auxin-responsive genes is up-regulated in response to low R:FR ratios (Devlin et al., 2003). As a loss of the shade-induced hypocotyl elongation response was observed in two of the four *spa* triple mutants and the *spa-Q*, the involvement of *SPA* genes in the regulation of the transcript levels of early shade marker genes was tested. To this end, seedlings were grown for four days in Wc and were subsequently shifted to Wc+FRc (low R:FR) conditions or kept in Wc for the indicated time and the transcript levels of the shade marker genes were determined (Fig II-3). A significant up-regulation of *HFR1*, *ATHB-2* and *PIL1* in the WT was observed as early as 30 minutes after the onset of the shade treatment, the earliest time-point tested in this study (data not shown). Transcript levels of the shade marker genes *ATHB2*, *PIL1* and *XTR7* increased strongly in simulated shade conditions after three hours compared with Wc in the WT (Fig. II-2 A). The transcript levels of *ATHB*2 and *PIL1* equally increased in all *spa* multiple mutants in low R:FR compared to the WT. This indicates that *SPA* genes are not required for the initial accumulation of these transcripts in response to shade.

The transcript levels of *XTR7* were found to be significantly lower in all multiple *spa* mutants in low R:FR conditions compared with the WT and the induction of the *XTR7* transcript level by low R:FR was weaker. The highest induction of the transcript level of *XTR7* in the *spa* mutants was found in the *spa1 spa2 spa3* mutant that exhibited a significantly higher induction compared to all other *spa* mutants (Fig. II-3 A). This indicates that the full induction of *XTR7* by simulated shade requires functional *SPA* genes. It also provides a correlation between the aberrant elongation phenotypes of the *spa* multiple mutants compared to the WT and the transcript levels of a gene directly involved in the elongation of cells, except for the results obtained with the *spa2 spa3 spa4* mutant (Fig II-2 B; Fig. II-3 A).

The transcript levels of another shade marker gene, *IAA19,* were also determined in the WT and the *spa1 spa3 spa4* mutant in response to simulated shade treatment. The induction of the *IAA19* transcript level was equal in WT and the *spa1 spa3 spa4* mutant, though the *IAA19* transcript exhibited lower levels in the *spa1 spa3 spa4* mutant in both conditions compared with WT. This indicates that the overall transcription levels of *IAA19,* but not the induction in response to low R:FR conditions, depend in part on *SPA* gene function (see also figure II-24).

To test, if *SPA* genes might be required for the induction of transcript levels of shade marker genes in the seedling after prolonged shade treatment, the transcript levels of the shade marker

HFR1 were analysed after 24 and 48 hours of simulated shade treatment and Wc in the *spa1 spa3 spa4* triple mutant and the *cop1-4* mutant (Fig. II-3 B). *HFR1* transcript levels were found to be strongly increased in simulated shade in the WT and both mutants after 24 and 48 hours. This indicates that neither *SPA* genes, nor *COP1* are limiting for the up-regulation of *HFR1* transcript levels in shade avoidance. This is in agreement with the previous observation that the regulation of *HFR1* transcript levels in response to low R:FR conditions is largely independent of *COP1* (Crocco et al., 2010).

PIL1 is rapidly and strongly up-regulated by shade conditions. Furthermore, *PIL1* levels remain elevated in prolonged shade. The transcript levels of *PIL1* were also analysed in prolonged shade in a time-course experiment (Fig. II-3 C). The *spa1 spa3 spa4* and the *cop1-4* mutants showed strongly increased transcript levels of *PIL1* in response to low R:FR conditions that compare to the induction in the WT. The *PIL1* transcript levels in Wc remained low in all lines. This suggests that *SPA* genes and *COP1* are not limiting for the maintenance of high transcript levels of *PIL1* and presumably other shade marker transcripts (data *ATHB2* not shown) in prolonged shade conditions. The results of this study contradict the proposed function of *COP1* in the general regulation of early shade marker genes, as *COP1* was observed to negatively act on the increase of *ATHB2* and *PIL1* transcript levels in response to a low R:FR treatment (Roig-Villanova et al., 2006). On the other hand, *PIL1* transcript levels increased similarly in the WT and *cop1* mutant in short term shade previously (Crocco et al., 2010), which is in agreement with this study.

II.1.1.3 *SPA* gene function in adult leaves in low R:FR conditions

SPA genes regulate adult stages in plant development, influencing final leaf size and also the timing of flowering (Laubinger et al., 2004 and 2006; Fittinghoff et al., 2006; Fackendahl, PhD Thesis, 2011 Ranjan et al., 2011). Hence, it was tested, if *SPA* genes are involved in the control of adult plant growth in response to our simulated shade conditions. Plants were grown on soil for four days in Wc and moved to simulated shade or kept in Wc for additional seven days (Fig. II-4). The elongation response to low R:FR conditions of the leaf petiole was detected in the WT that exhibited longer petioles, but leaf blades of similar size compared with Wc conditions (Figure II-4 B). The petioles from all *spa* triple mutants upheld robust responsiveness to the low R:FR treatment, while neither *spa-Q* nor *cop1-4* showed any elongation response of the true leaf petioles. These results suggest that *SPA* genes and *COP1* have an essential function in the elongation response of leaf petioles to low R:FR conditions. In contrast to the seedling phenotype, where *SPA* genes differentially contribute to the

elongation response of hypocotyls and petioles of cotyledons, no qualitative difference in the *SPA* gene function could be found in the elongation response of petioles of true leaves in response to FR enriched conditions.

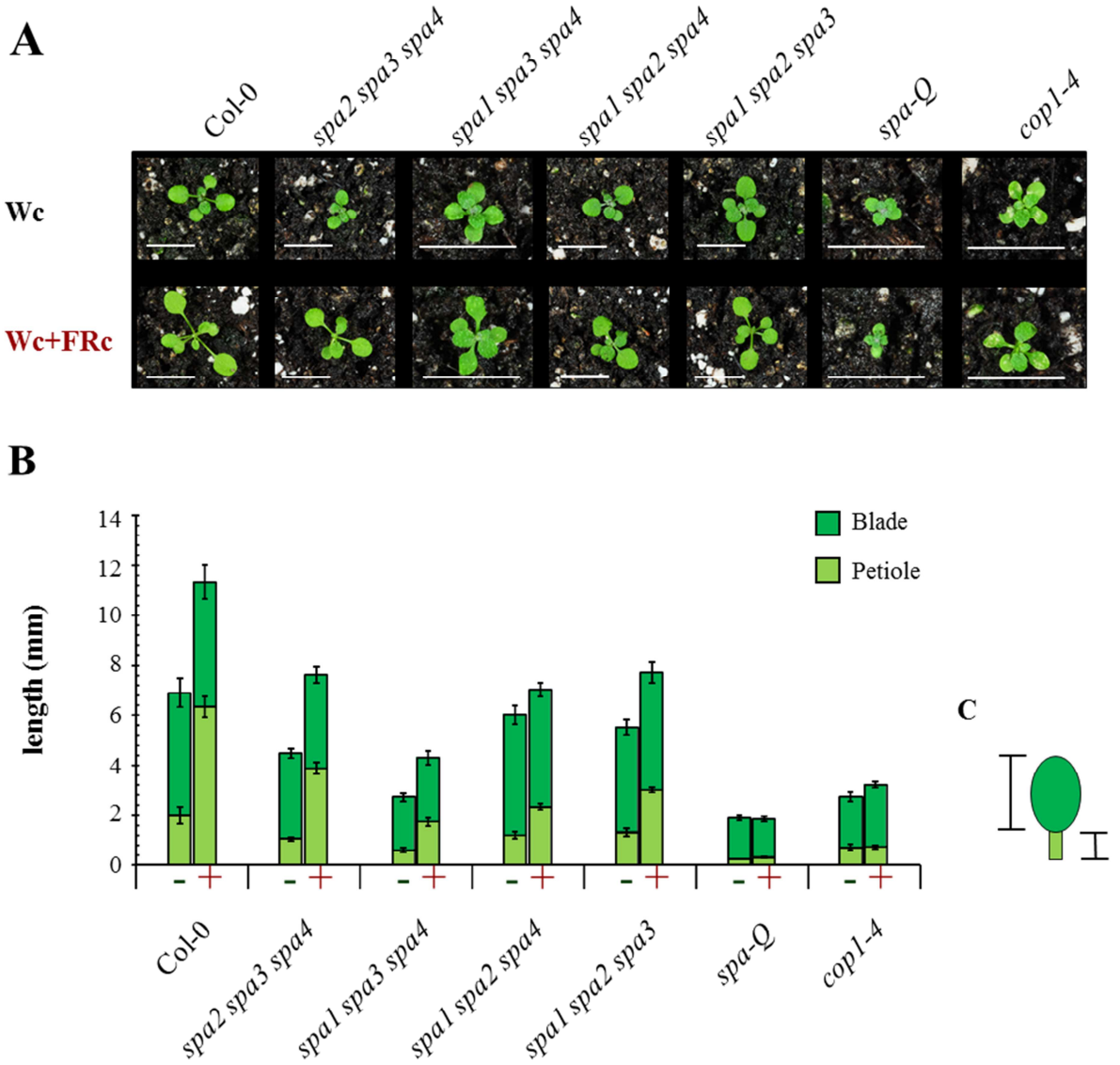

Figure II-4: Adult plant growth in response to low R:FR is regulated by SPA genes and COP1. A) Pictures of 11-day-old plants grown in continuous white light (Wc) or shifted to Wc supplemented with continuous far-red light (Wc+FRc) after four days**. B)** Leaf length measurements. The total leaf length and the petiole length of the longest leaves of plants grown in the Wc (-) or Wc+FRc (+) conditions were measured for each genotype and the leaf blade length calculated by substraction of the petiole length from the total leaf length. Data is represented as mean of blade length (± SEM of total length) and petiole length ± SEM, n > 8. **C)** Sketch of the two values represented in **B)**, the blade (dark green) and the petiole (light green) of a true leaf.

II.1.1.4 Accelerated flowering in low R:FR is independent of *SPA* and *COP1* genes

Shaded Arabidopsis plants react by accelerating flowering in long day conditions (Wollenberg et al., 2008). *COP1* and *SPA1* have a function in the photoperiodic pathway of flowering time control, as they inhibit early flowering in short days (Laubinger et al., 2006; Jang et al., 2008). Thus, the flowering time of all *spa* triple mutants and the *spa-Q* and *cop1-4* mutants were determined in Wc and low R:FR light after four days of initial development in Wc (Fig. II-5).

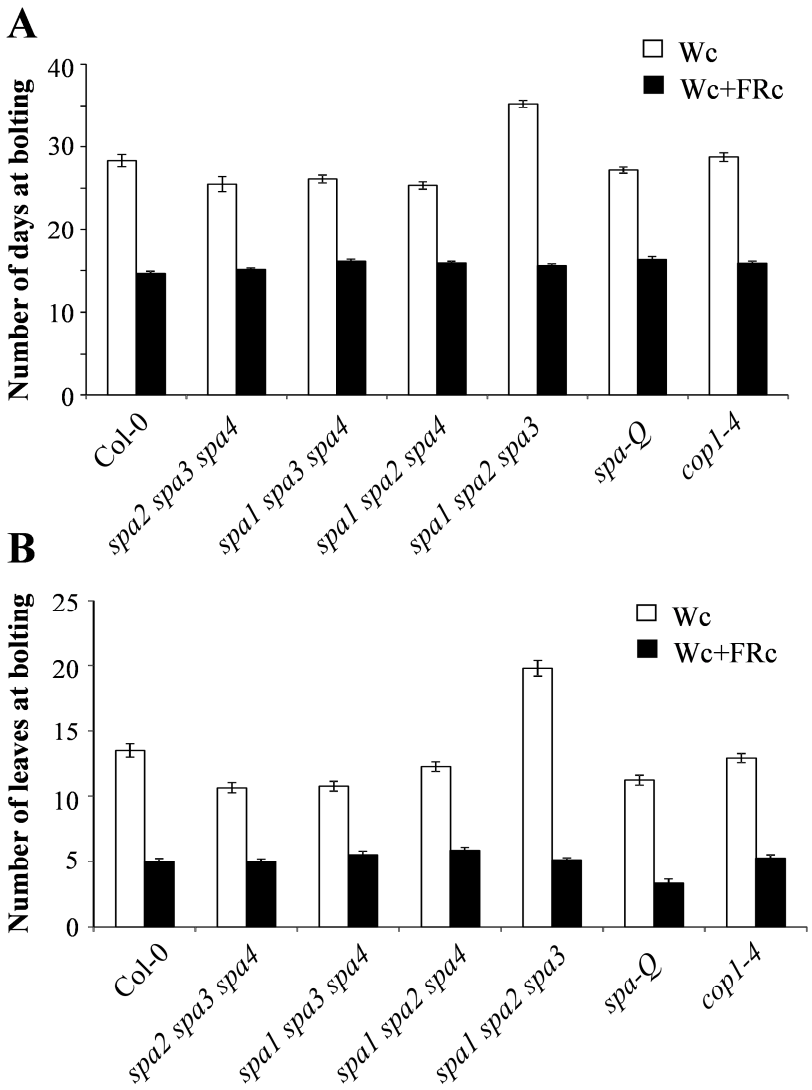

Figure II-5: Acceleration of flowering time in response to low R:FR is independent of *SPA* genes and *COP1*. The number of days **(A)** and number of true leaves **(B)** at bolting counted from plants grown on soil in continuous white light (Wc) or Wc supplemented with continuous far-red light (Wc+FRc) conditions after four days of Wc treatment. Data represented as mean of the flowering time of single plants (n≥8). Error bars represent SEM.

The WT plants incubated in low R:FR conditions bolted markedly earlier and with fewer leaves compared with the plants grown in Wc (Fig. II-5 A,B). The flowering time in Wc of all

spa triple and quadruple mutants, except the *spa1 spa2 spa4* mutant was indistinguishable from the WT. The *spa1 spa2 spa3* mutant was found to flower significantly later in Wc conditions than the WT or any other *spa* mutant or the *cop1-4* mutant. This was supported by observations in flowering time experiments in long day conditions (P. Fackendahl, personal communication). All genotypes displayed an acceleration of flowering time in simulated shade for both parameters similar to the WT flowering time.

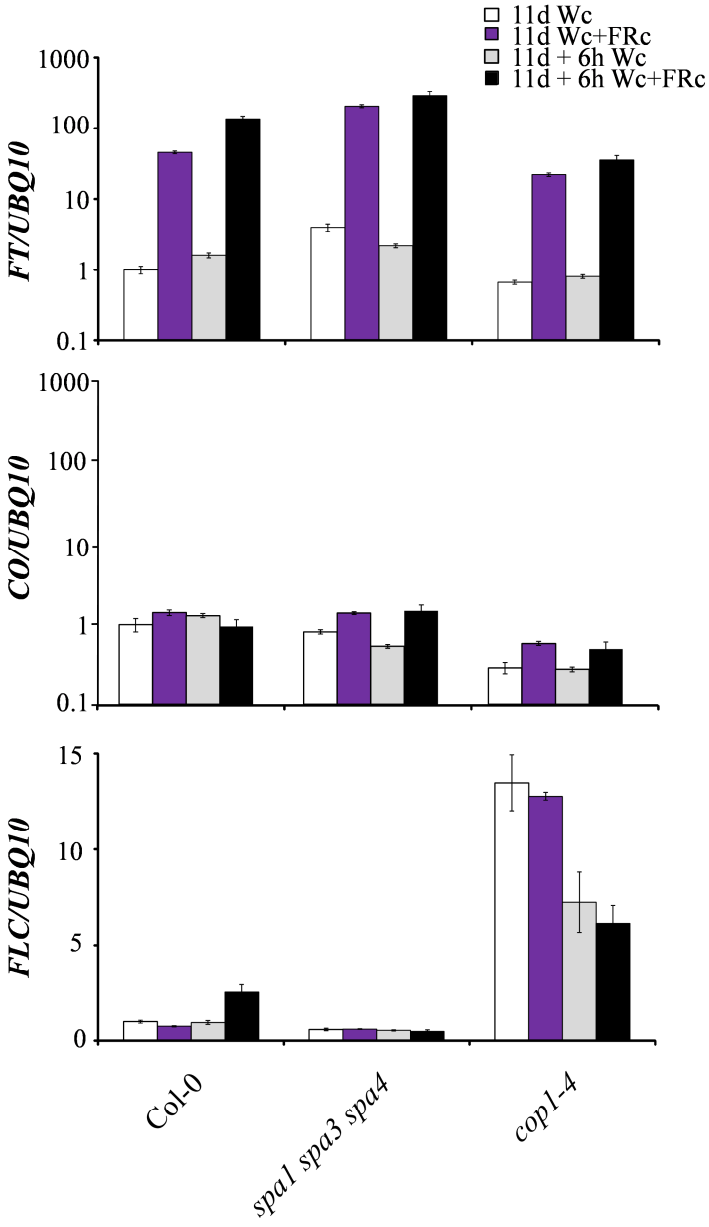

Figure II-6: The regulation of flowering time control genes in low R:FR conditions by *SPA* genes and *COP1*. The transcript levels of the floral integratos *FT*, *CO* and *FLC* were analysed in WT, *spa1 spa3 spa4* and *cop1-4* seedlings grown for four days in continuous white light (Wc) and shifted to Wc supplemented with far-red light (Wc+FRc) for additional seven days or kept in Wc. At a second time-point (6 h later), seedlings were harvested from both conditions. *UBQ10* was used as endogenous control. Data represent the mean of three biological replicates ± SEM.

This result shows that neither *SPA* genes nor *COP1* are involved in the acceleration of flowering in our simulated shade conditions. This indicates that phyB causes the acceleration of flowering time in low R:FR conditions independently of the COP1/SPA complex which is an important player in the photoperiodic flowering pathway in high R:FR (Laubinger et al., 2006).

In order to investigate the gene regulation of floral inducers and repressors implicated in the acceleration of flowering in low R:FR, the transcript levels of *FT*, *CO* and *FLC* were determined in the WT, *spa1 spa3 spa4* and the *cop1-4* mutant using qRT-PCR (Fig. II-6). The transcript levels of *FT* were strongly elevated in the WT background at the two chosen time-points in low R:FR grown plants compared with Wc grown plants. This elevation was also observed in the *spa* triple and the *cop1-4* mutant. This indicates that *FT* accumulates in response to the low R:FR treatment independently of *SPA* or *COP1* genes and correlates with the observed flowering time phenotypes.

CO transcript levels in the WT were similar at both time-points and in both conditions, but increased slightly in the *spa1 spa3 spa4* mutant and the *cop1-4* mutant in response to low R:FR conditions compared with Wc. *FLC* levels were unresponsive to the low R:FR treatment in all backgrounds, but the levels were low in the WT and the *spa1 spa3 spa4* mutant and highly elevated in the *cop1-4* background. The elevated *FLC* levels in the *cop1-4* background correlate with the overall lower transcript levels of *FT*, but the *FT* transcript levels in the *cop1-4* mutant were induced in the low R:FR conditions independent of the higher *FLC* transcript levels. The increased levels of *FLC* transcript in the *cop1-4* mutant should be subject to further investigation with additional *cop1* mutants. Taken together, *FT* levels were elevated in response to low R:FR independently of the *CO* and *FLC* transcript levels in all backgrounds.

II.1.2 Analysis of the distinct functions of *SPA1* and *SPA2* in the regulation of the SAS

II.1.2.1 SPA1/SPA2 promoter-swap analysis

SPA1 sustains a hypocotyl elongation in response to low R:FR compared to Wc in the *spa2 spa3 spa4* mutant, whereas *SPA2* could not serve this function in the *spa1 spa3 spa4* mutant (Figure II-2). This difference in *SPA1* function compared with *SPA2* could be due to sequence differences in the protein-coding sequences or the regulating regions. The overlapping and distinct functions of *SPA1* and *SPA2* in photomorphogenesis were previously investigated using a promoter-swap approach (Balcerowicz *et al.*, 2011; Fittinghoff, PhD Thesis, 2009).

Transgenic lines expressing SPA1 or SPA2 from the *SPA1* or *SPA2* regulatory sequences (5' and 3' regulatory regions) in a *spa-Q* mutant were analysed for complementation of the shade dependent elongation of the hypocotyl (Figure II-7). All lines that expressed the SPA1 protein over-complemented the *spa-Q* mutant hypocotyl shade phenotype. As observed previously, also the white light phenotype is at least fully complemented in all SPA1 expressing lines (Fittinghoff, PhD Thesis, 2008). Driven by the *SPA2* promoter, SPA2 did not cause over-complemention of the elongation response.

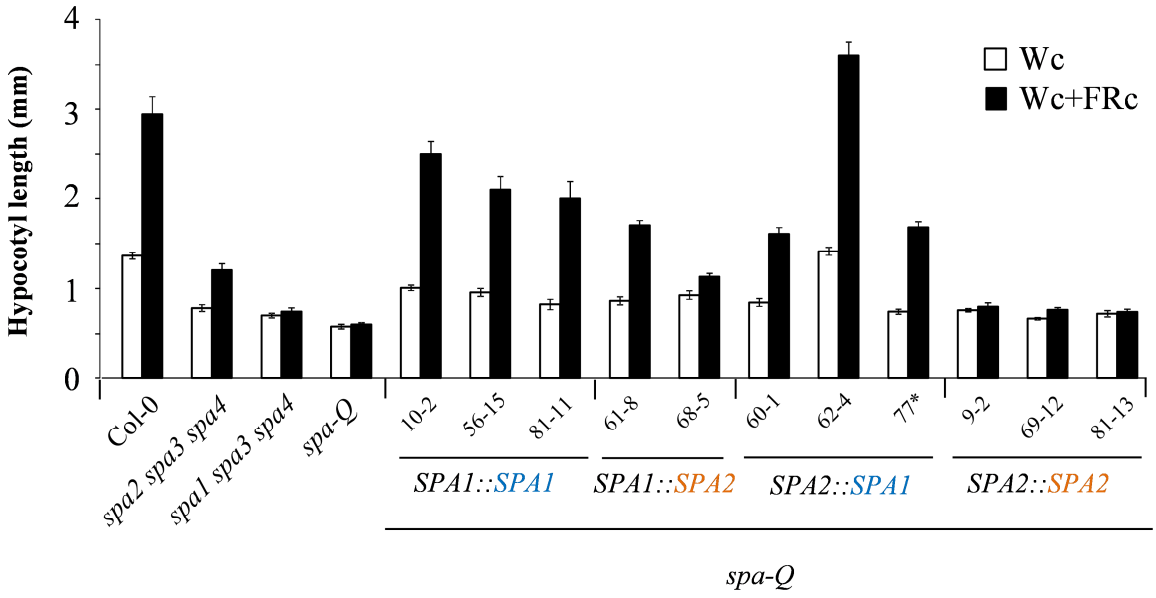

Figure II-7: Divergent function of *SPA1* and *SPA2* in low R:FR derives from a combination of the regulatory sequences and the protein coding sequences. Hypocotyl measurements of black MS grown seedlings are presented. Lines expressing SPA1 or SPA2 from the 5' and 3' regulatory regions of *SPA1* or *SPA2* (promoter-swap constructs described in Balcerowicz et al., 2011 and Fittinghoff, PhD Thesis, 2009). Seedlings were grown in continuous white light (Wc) for 6 days or shifted to Wc supplemented with far-red light (Wc+FRc) after 3 days (≥ 10 seedlings were measured per genotype and condition, data presented as mean ± SEM)). The asterisk (*) indicates a still segregating line.

However, when expressed from the *SPA1* regulatory sequences, the SPA2 protein caused an elongation of the hypocotyl in response to low R:FR compared to the Wc conditions at least in one transgenic mutant line (in a second independent experiment, *SPA1::SPA2* #68-5 also exhibited a more pronounced hypocotyl elongation similar to #61-8, data not shown). Thus, *SPA2* alone is able to elicit the shade avoidance response in a *spa1 spa2 spa3 spa4* quadruple mutant, but only if SPA2 is under the control of *SPA1* regulatory sequences. This indicates that the protein sequence and the promoter activity both contribute to the distinct function of *SPA1* and *SPA2* in the low R:FR triggered elongation response of seedlings.

II.1.2.2 Analysis of *SPA* transcript levels in response to low R:FR

As *SPA* genes are important for the hypocotyl elongation in response to low R:FR conditions, it is conceivable that their expression would be shade-regulated. Moreover, differential regulation of the four *SPA* genes might contribute to their difference in function. As the transcript levels of *SPA1*, *SPA3* and *SPA4* are light-induced, it could be expected that the supplemental FRc in the low R:FR conditions might result in elevated transcript levels of these genes (Hoecker et al., 1999; Fittinghoff et al., 2006).

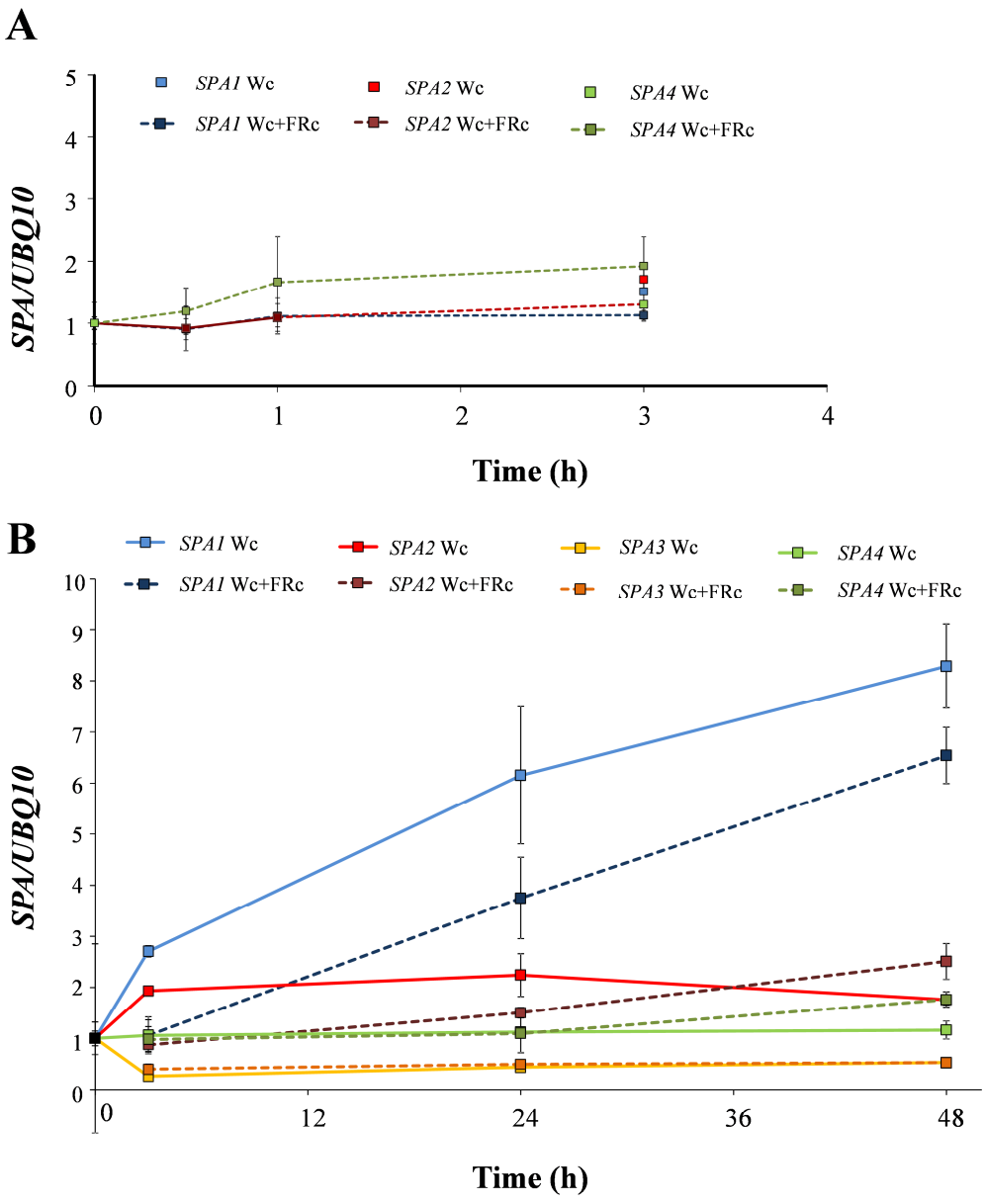

Figure II-8: Regulation of *SPA* transcript levels in response to low R:FR conditions. Relative transcript levels of the four *SPA* genes were determined by qRT-PCR. Seedlings were grown for four days in Wc and transfered to low R:FR for the indicated time or kept in Wc. *UBQ10* was used as endogenous control. Data were calibrated to Col-0 0 h for each gene and shown as the mean of three biological replicates ± SEM. **A)** Short-term experiment between 0 h and 3 h **B)** Long-term experiment between 0 h and 48 h.

The transcript levels of the *SPA* genes were determined in seedlings after different durations of Wc+FRc treatment (Fig. II-8). Thirty minutes after the onset of the shade treatment, the *SPA* transcript abundance was similar to the Wc conditions, thus the *SPA* transcript levels were not regulated in short-term shade (Fig. II-8 A). Also, longer shade exposure did not result in an increased difference in *SPA* transcript levels between Wc and low R:FR grown seedlings (Figure II-8 B). *SPA1* transcript levels increased over time in both simulated shade and Wc conditions, presumably indicating developmental dependent gene regulation. These data indicate that *SPA* transcript levels are not regulated by low R:FR conditions. Hence, the difference in SPA function is likely determined by differential activity of the SPA proteins.

II.1.2.3 Analysis of SPA1 protein level in response to low R:FR

SPA protein levels are subject to regulation in response to light signals (Balcerowicz et al., 2011). Thus, stabilisation of SPA1 could contribute to its activity in the elongation responses to low R:FR. To determine the protein levels of SPA1 in Wc-grown seedlings and seedlings shifted to low R:FR conditions, an immunoblot with a SPA1-specific antibody was performed (Fig. II-9). The total protein levels in all samples were comparable as indicated by the overall equal tubulin levels. The SPA1 signal was absent from the *spa1-100* null mutant and strong in the SPA1 overexpressing line (*SPA1::SPA1-HA* in RLD; Fittinghoff et al., 2006), though a faint background band was repeatedly observed in the null mutant.

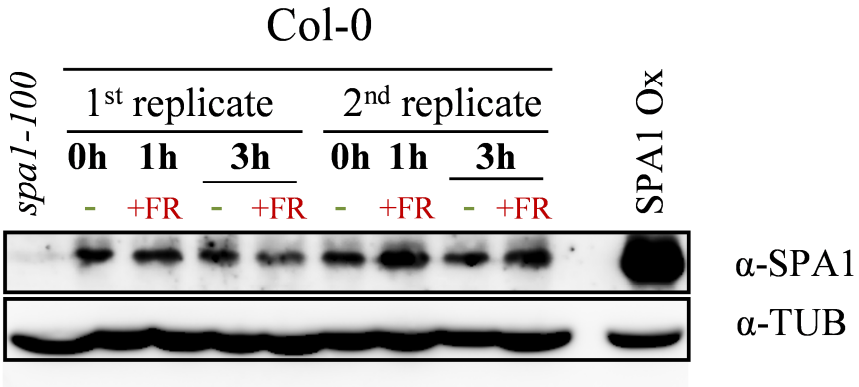

Figure II-9: SPA1 protein levels in simulated shade. Immunodetection of SPA1 protein levels in 4-day-old WT seedlings grown in continuous white light (Wc) and shifted to simulated shade for the indicated time (+FR) or kept in Wc (-). SPA1 was detected with a SPA1 specific antibody (Maier, PhD Thesis, 2011). Tubulin levels were detected as loading control. Forty µg of total protein extract were loaded. For each time-point and light condition, two biological replicates are shown.

The SPA1 levels in the protein samples taken from Wc and Wc+FRc treated seedlings showed no difference after one or three hours of the treatment. 24 and 48 hour time-points were also analysed with the same trend (data not shown). This indicates that the SPA1 protein

levels are not altered in response to the low R:FR treatment. Nevertheless, differences in SPA2 protein levels in Wc and simulated shade may contribute to the distinct function of *SPA1* and *SPA2* in the elongation responses of seedlings to low R:FR treatment. However, this question was not addressed as nuclear preparations would be necessary to determine SPA2 levels, which was not attempted due to time restriction.

II.1.3 *SPA* genes genetically interact with *HFR1*, but not *HY5* in shade avoidance

The COP1/SPA complex acts as a negative regulator of transcription factors that trigger light responses (reviewed in Hoecker, 2005). Among these, HFR1 serves a prominent function as a negative regulator of shade avoidance responses (Sessa et al., 2005; Hornitschek et al., 2009). Overexpression of the HFR1 protein leads to a reduced hypocotyl elongation response to low R:FR conditions (Hornitschek et al., 2009; Galstyan et al., 2011). Thus, the question was addressed, whether the *SPA* genes might act via *HFR1* to function in shade avoidance. Preliminary data in this regard were already obtained in our group under my supervision (Sahm, J., Examensarbeit 2010). The *hfr1* mutant, *spa* mutants and a *spa hfr1* quadruple mutant were used for the genetic interaction study and seedlings grown as described in figure II-2. In accordance with its function as a negative regulator of shade avoidance, *hfr1-101* mutants exhibited a longer hypocotyl only in low R:FR conditions compared to the WT (Figure II-10 A,B). As seen earlier in this study, the triple mutant only expressing the SPA2 protein (*spa1 spa3 spa4*) did not show a shade avoidance phenotype. However, the introduction of *hfr1-101* into this background partly restored the elongation response. This indicates that *SPA* genes interact with *HFR1* in the response of the hypocotyl to low R:FR conditions and suggests that *SPA* genes act positively on shade avoidance by the repression of negative regulators as proposed for *COP1* earlier (Crocco et al., 2010). It was reported that HFR1 inhibits transcript over-accumulation of *ATHB2* as early as one hour after the onset of low R:FR conditions (Sessa et al., 2005). To investigate the regulation of *ATHB2* in response to our shade avoidance conditions, the transcript levels were determined in the *hfr1-101* mutant and the *hfr1 spa1 spa3 spa4* quadruple mutant after three hours of simulated shade treatment (Fig. II-10 C). No over-accumulation of the transcript was observed in the *hfr1* mutant, thus the reported transcript levels in the *hfr1* mutant background could not be confirmed (Sessa et al., 2005). When compared to the WT, *ATHB2* transcript levels were equally up-regulation within 3 hours of low R:FR treatment compared with Wc. Also, in the *spa* triple mutant background, the induction of the *ATHB2* level was comparable to the WT, though lower levels were observed in Wc conditions.

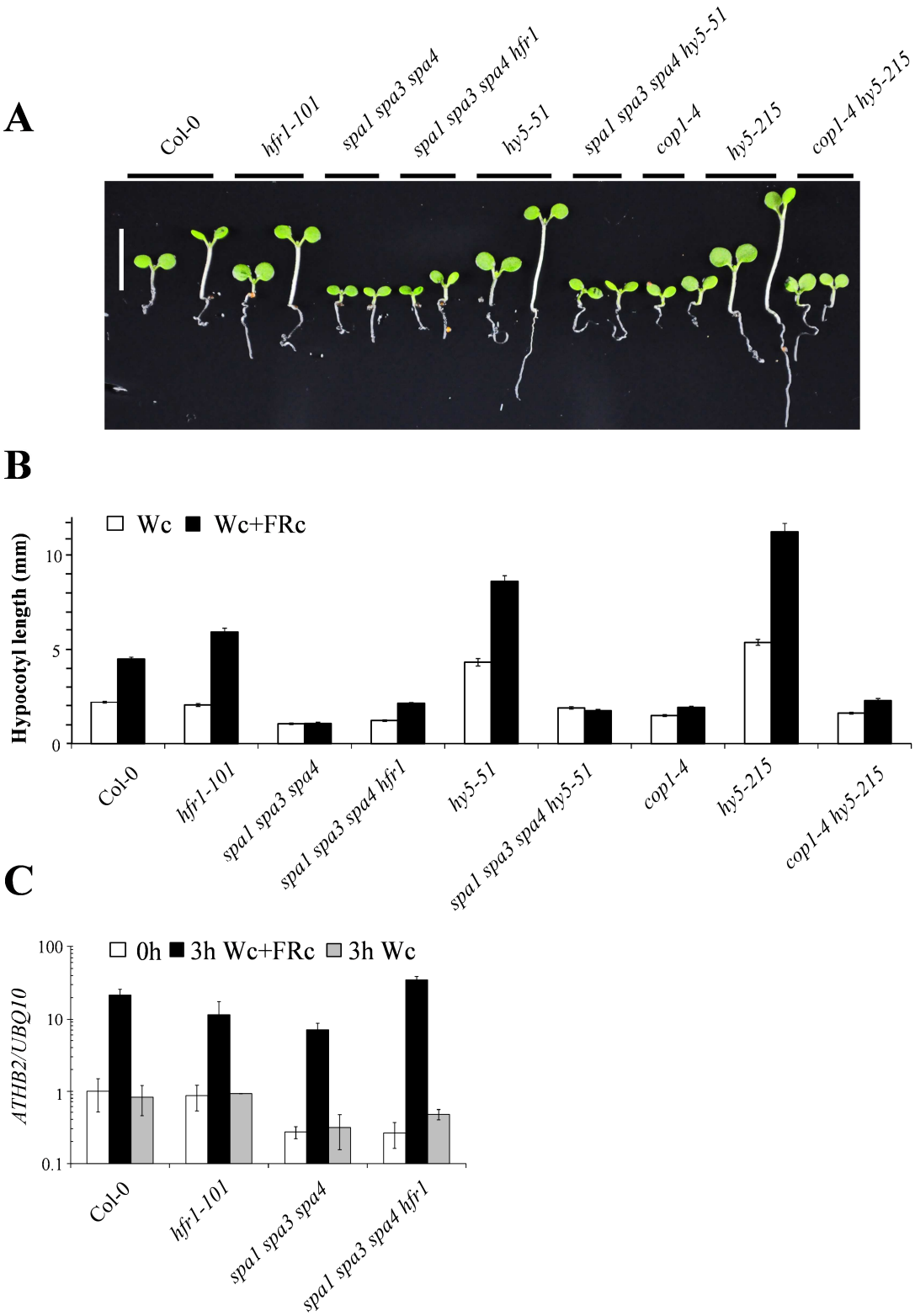

Figure II-10: *hfr1*, but not *hy5* mutation can rescue the hypocotyl elongation response to low R:FR in a *spa* triple mutant background. A) Continuous white light (Wc) and simulated shade (Wc supplemented with far-red light; Wc+FRc) phenotypes of eight-day-old seedlings. Within each pair of seedlings of one genotype, Wc grown seedlings are on the left, seedlings grown in low R:FR on the right. The white bar represents 10 mm. **B)** Hypocotyl length of soil-grown mutants in Wc and Wc+FRc (>15 seedlings were measured, data presented as mean ± SEM)). **C)** Relative transcript levels of *ATHB2* were analysed with quantitative qRT-PCR. Seedlings were grown for 4 days in Wc and subsequently shifted to low R:FR conditions or kept in Wc for 3 h. *UBQ10* was used as endogenous control. Data represent the mean of three biological replicates ± SEM.

However, the *ATHB2* transcript levels increased stronger in the *spa1 spa3 spa4 hfr1* mutant background than in the *spa* triple mutant background. Thus, the up-regulation of the transcript levels of *ATHB2* was not limited by the *SPA* genes or *HFR1* alone in this experiment, but a genetic interaction between *SPA* genes and *HFR1* was suggested on the level of *ATHB2* regulation.

Another target of the COP1/SPA complex, HY5, has been associated with the shade avoidance response of seedlings recently (Sellaro et al., 2011). HY5 is a positive regulator of the sun-fleck response that is elicited in shaded plants that sporadically receive high R:FR signals, due to a changing lighting of the environment. Thus, altered regulation of HY5 in the *cop1-4* and the *spa1 spa3 spa4* triple mutant may cause shade avoidance related phenotypes and the elongation response of *hy5* mutants. Crosses of *hy5* with the *cop1-4* and the *spa1 spa3 spa4* mutants were analysed accordingly (Fig. II-10 A,B). Both *hy5* mutants exhibited longer hypocotyls in Wc, but normal elongation response compared with the WT. Furthermore, the *hy5* mutations were not able to restore the diminished elongation response to low R:FR of the *spa* triple mutant or the *cop1-4* mutant. Thus, *HY5* was not required for the elongation response to low R:FR conditions. Furthermore, no shade specific genetic interaction of *COP1* or the *SPA* genes was observed with *HY5*, while the exaggerated elongation of *hy5* mutants in white light was clearly *SPA* and *COP1* dependent as described previously (Osterlund et al., 2000; Saijo et al., 2003).

II.1.4 Genetic interaction of phytochrome photoreceptors with *SPA* and *COP1* genes in low R:FR

The COP1/SPA complex is a central regulator of light signalling that is a direct and indirect downstream target of a large portion of the photoreceptors. It also functions upstream of phyA and phyB by regulating the protein levels of these photoreceptors (Boccalandro et al., 2004; Jang et al., 2010; Lian et al., 2011; Liu et al., 2011; Seo et al., 2004; Wang et al., 2001; Yang et al., 2001). The function of the COP1/SPA complex in shade avoidance may thus be upstream or downstream of phyA and/or phyB, as both phytochromes regulate the elongation response antagonistically. The *spa1 spa3 spa4* mutant background was employed to analyse a genetic interaction of *SPA* genes with *PHYA* under low R:FR conditions (Fig. II-11). The *phyA-211* single mutant exhibited an exaggerated shade phenotype compared to the WT, while no difference was observed in Wc between *phyA-211* and the WT. The *spa1 spa3 spa4 phyA* quadruple mutant displayed a pronounced elongation response in low R:FR conditions compared to the *spa1 spa3 spa4* triple mutant. This may reflect the relief of a hyperactive

phyA-pathway in the *spa* triple mutant or may originate from a *SPA* independent phyA function.

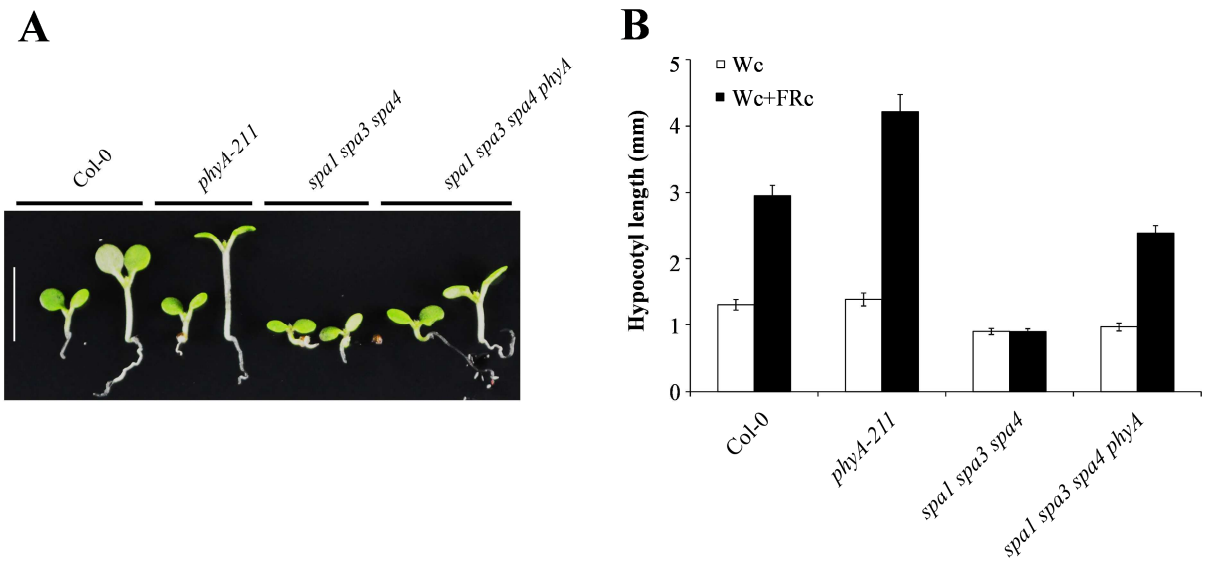

Figure II-11: Introduction of *phyA* mutation restores elongation response of spa1 spa3 spa4 to low R:FR.
A) Shade phenotype of six-day-old seedlings. Within each pair of seedlings of one genotype, seedlings grown in continuous white light (Wc) are on the left, seedlings grown for three days in Wc and shifted for three days to low R:FR, are on the right. The white bar represents 10 mm. **B)** Hypocotyl lengths of black MS grown mutants. Seedlings were grown under Wc for 6 days or shifted to white light supplemented with far-red light (Wc+FRc) after 3 days (>15 seedlings were measured per genotype, data presented as mean ± SEM).

A putative dependency of phyB signalling on *COP1* in low R:FR was tested with a *cop1-6 phyB* double mutant (Fig. II-12). The *cop1-6* single and the *cop1-6 phyB-9* double mutants exhibited shorter hypocotyls than the WT and the *phyB-9* mutant in both light conditions.

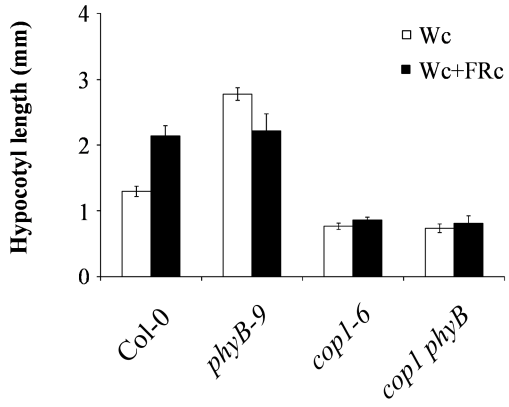

Figure II-12: *cop1* is epistatic over *phyB* in Wc and low R:FR. Hypocotyl length measurements of black MS grown WT (Col-0), *phyB-9*, *cop1-6* and *cop1 phyB* double mutants. Seedlings were grown in continuous white light (Wc) for six days or shifted to Wc supplemented with continuous far-red light (Wc+FRc) after 3 days (n≥15, data presented as mean ± SEM).

Furthermore, the hypocotyl was not responsive to the low R:FR treatment in the *cop1-6* and the *cop1-6 phyB-9* double mutant, that both exhibited similar hypocotyl lengths in both light conditions. This result shows that *cop1-6* suppresses the constitutive *phyB* phenotype completely, indicating that *cop1* is epistatic over the *phyB* mutation in both light conditions.

Notably, the *spa1-2* mutation is capable of completely suppressing the *phyB* mutant phenotype of adult leaf blades in a *spa1-2 phyB-1* double mutant (Ranjan et al., 2011) indicating an essential SPA1 function downstream of phyB involved in the constitutive shade avoidance phenotype of the adult plant. Thus, it was analysed, if *spa1* was also epistatic over *phyB* in seedlings incubated in simulated shade (Fig. II-13).

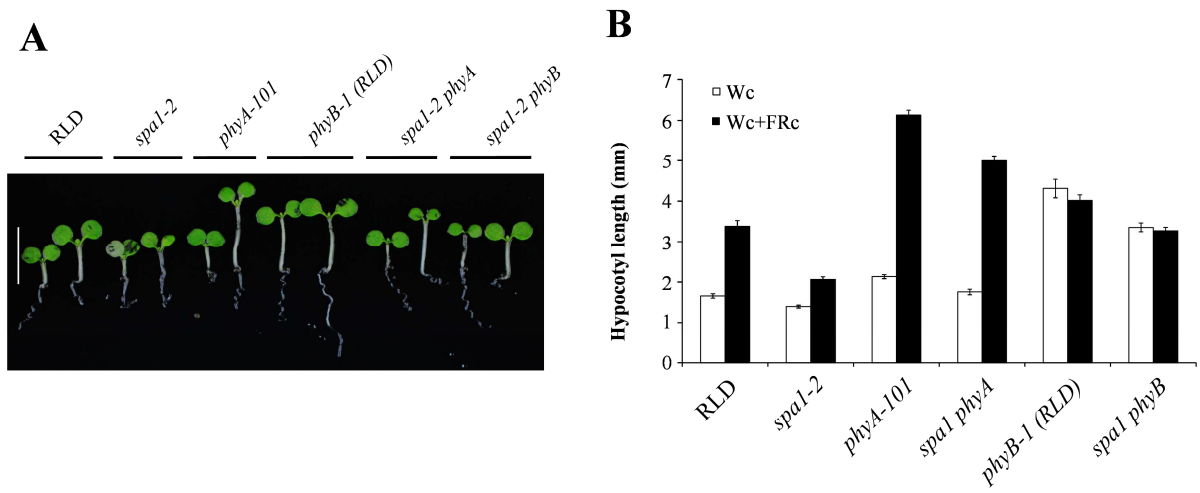

Figure II-13: Genetic interaction analysis of the *spa1-2* single mutant with *phyA* and *phyB* in low R:FR. A) White light (Wc) and shade phenotype of six-day-old seedlings grown on black MS in Wc (left) or shifted to low R:FR after three days (right). The white bar represents 5 mm. **B)** Hypocotyl lengths of black MS grown mutants. Seedlings were grown in Wc for six days or shifted to Wc supplemented with far-red light (Wc+FRc) after 3 days (>15 seedlings were measured per genotype, data presented as mean ± SEM).

Unlike the *spa1-7* mutant (Col-0 background), the *spa1-2* single mutant exhibited a significantly reduced hypocotyl elongation in response to low R:FR conditions compared with the RLD wild type (Fig. II-13 B). The *phyA* mutant exhibited an increased elongation in response to the simulated shade. In the *spa1 phyA* double mutant, the reduction of the shade phenotype of the *spa1-2* single mutant was reversed, as the mutant exhibited an exaggerated shade dependent elongation response compared with the *spa1-2* mutant or the RLD WT.
The hypocotyl length of the *spa1-2 phyB-1* double mutant was reduced in white light and simulated shade conditions compared to the *phyB-1* mutant, but still considerably elevated compared to the *spa1-2* single mutant.

Taken together, these results indicate that *SPA1* contributes to the elongation response to low R:FR conditions and that phyB partly depends on *SPA1*, but to a far lesser extent than what was previously observed for the adult leaf phenotype (Ranjan et al., 2011).

II.1.5 Structure-function analysis of SPA4 in low R:FR

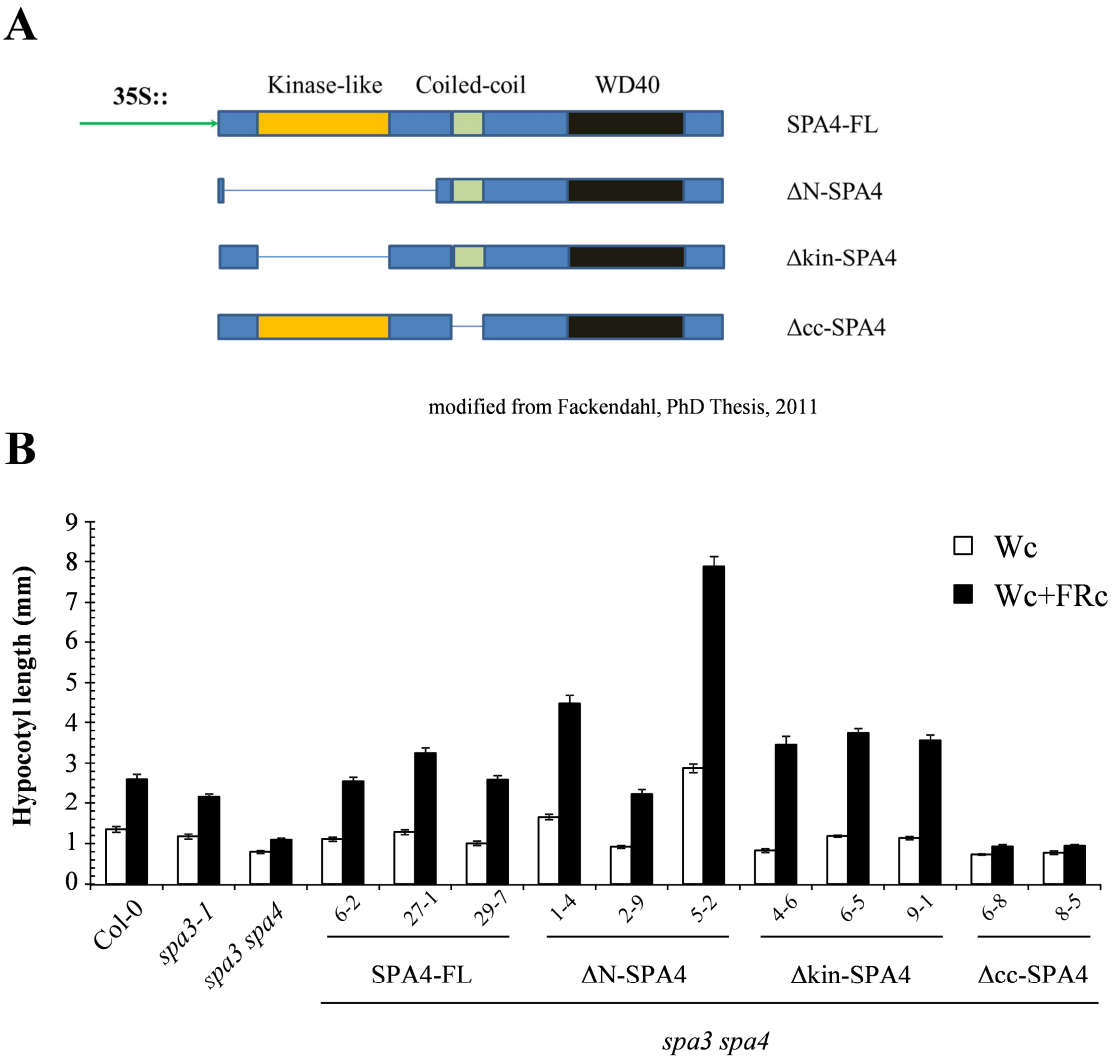

Figure II-14: The N-terminal domain of SPA4 is not limiting for the hypocotyl elongation response to low R:FR. A) Domain structure of the SPA4 protein and of the SPA4 deletions expressed in the *spa3-1 spa4-1* double mutant background. Lines were described earlier (Fackendahl, PhD thesis, 2011) **B)** Hypocotyl length measurements of black MS grown mutants. Seedlings were grown in continuous white light (Wc) for six days or shifted to Wc supplemented with far-red light (Wc+FRc) after three days (> 15 seedlings were measured per genotype and conditions, data presented as mean ± SEM).

SPA4 (alongside *SPA1*) has a function in shade avoidance related elongation responses, as supported by the *spa1 spa2 spa3* triple mutant phenotype in this study (see figure II-2). The investigation of the contribution of the different domains of SPA4 to the elongation response could promote the understanding of the function of SPA4 in shade avoidance. SPA proteins

contain a conserved coiled coil (cc)-domain for interaction with COP1 and other SPA proteins, a C-terminal WD-40 domain for substrate recognition and a more variable N-terminal domain that carries a kinase-like domain of yet unknown function (Figure II-14 A). The N-terminus of SPA1 has been found to be important for SPA1 function in seedlings and flowering time control (Fittinghoff, PhD Thesis, Fackendahl, PhD Thesis; Dieterle, personal communication).

In order to unravel functions of different SPA4 domains in the elongation response of seedlings, lines that express truncated protein versions of SPA4 driven by the *35S* promoter were tested for complementation of the *spa3 spa4* mutant phenotype (Fackendahl, PhD Thesis, 2011). Except the Δcc-SPA4 construct (SPA4 lacking the coiled-coil domain) that showed no complementation of the hypocotyl elongation, neither in Wc, nor in response to simulated shade, all lines fully complemented or over-complemented the hypocotyl elongation in response to low R:FR conditions (Figure II-14 B).
Here, making use of lines expressing different deletion constructs of SPA4, it was shown that the coiled-coil domain of SPA4 was necessary for SPA4 function in shade avoidance, suggesting that complex formation of COP1 and SPA4 and presumably with other SPA proteins is important for SPA4 function. Neither the kinase-like domain, nor the entire N-terminal domain of SPA4 is likely to serve a limiting function for the elongation response.
The fact that over-expression of SPA4 by the *35S* promoter leads to an elevated hypocotyl elongation in low R:FR indicates a dose-dependent activity of SPA4. Moreover, the function of *SPA4* is not dependent on regulation of the endogenous *SPA4* promoter in shade conditions.

II.1.6 Expression of SPA1 from the epidermis-specific *ML1* and *CER6* promoters rescues the *spa1 spa3 spa4* response to low R:FR

Functional *SPA1* is sufficient to maintain a pronounced shade avoidance response of the hypocotyl in the *spa2 spa3 spa4* triple mutant (Figure II-2). SPA1 is ubiquitously expressed throughout the seedling at high levels, but expression in the phloem is sufficient for its function in dark- and light-grown seedlings and in flowering time control (Fittinghoff et al., 2006; Ranjan *et al.*, 2011). In order to elucidate a tissue-specific *SPA1* function in the shade avoidance response of seedlings, lines expressing SPA1 from various tissue-specific promoters in the *spa1 spa3 spa4* mutant were analysed for complementation of the hypocotyl elongation phenotype (Figure II-15) (Ranjan *et al.*, 2011).

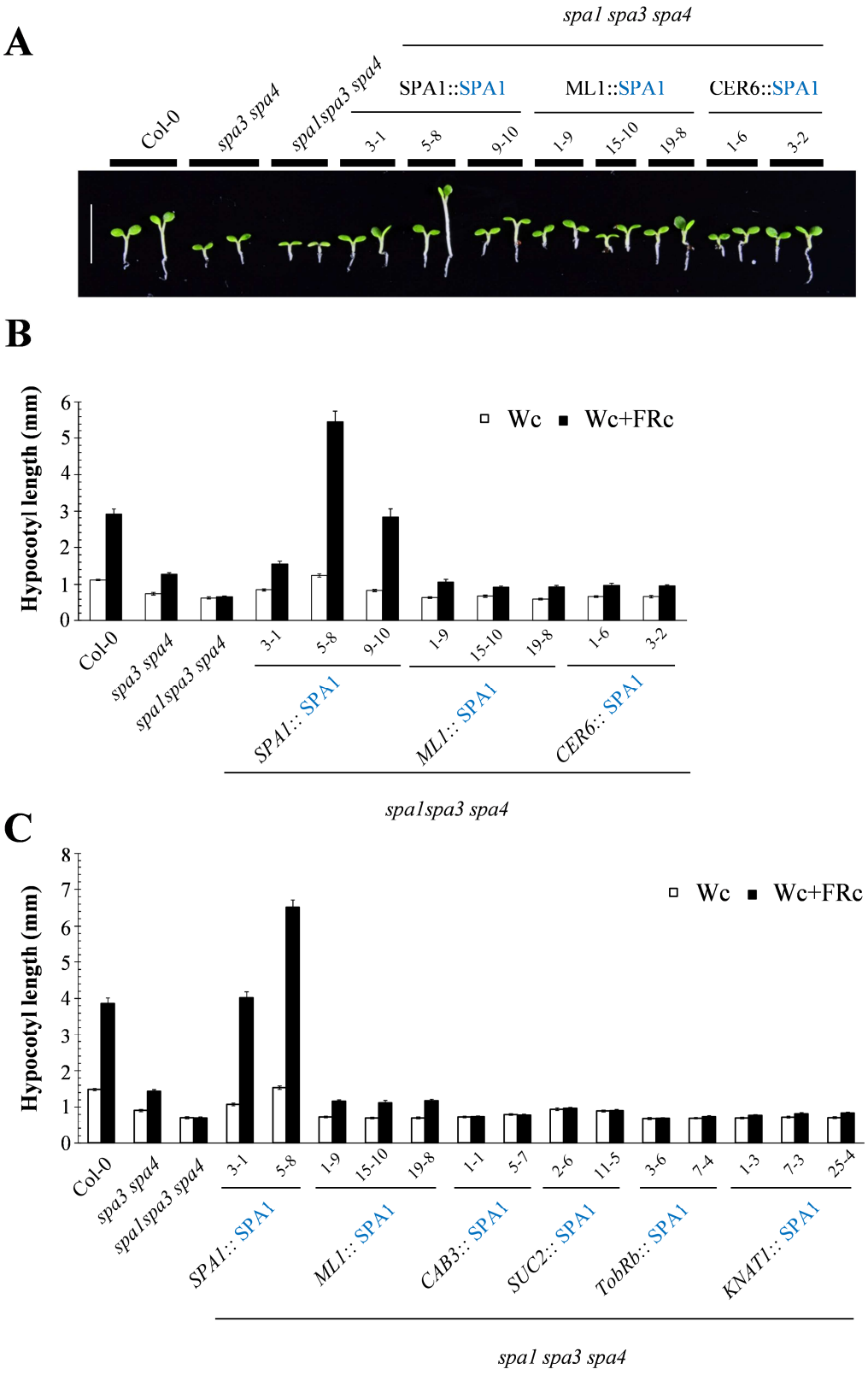

Figure II-15: Expression of SPA1 from two epidermis-specific promoters (*ML1* and *CER6*) restores the elongation response of the hypocotyl to low R:FR. Lines expressing SPA1 from various tissue-specific promoters were described earlier (Ranjan et al., 2011). **A)** White light (Wc) and shade phenotype of six-day-old seedlings grown on black MS in Wc (left) or shifted to low R:FR after three days (right). The white bar represents 5 mm. **B)** Hypocotyl length measurements of black MS grown mutants. Seedlings were grown in Wc for six days or shifted Wc supplemented with far-red light (Wc+FRc) after three days (n > 15, data presented as mean ± SEM)).

The tissue-specificity of the SPA1 expression in this *spa* mutant background has not been analysed, but differential expression of the promoters is assumed. *SPA1* over-complemented the mutant shade avoidance phenotype, when expressed from the endogenous *SPA1* promoter, as it was observed with *SPA1::SPA1* lines in the *spa-Q* background, analysed in this study (Figure II-15 A,B,C see also figure II-7).When SPA1 was expressed from the *ML1* promoter or the *CER6* promoter (epidermis-specific promoters), the elongation response to low R:FR was partly restored in all transgenic lines. This suggests a function for *SPA1* in the epidermis that leads to an elevated elongation of the hypocotyl in low R:FR conditions. The expression of *SPA1* in the shoot apical meristem (*KNAT1::SPA1*), the phloem (*SUC2::SPA1*), the root (*TobRB7::SPA1*) or the mesophyll (*CAB3::SPA1*) did not restore the hypocotyl elongation in low R:FR compared to Wc, while slightly longer hypocotyls were observed for lines expressing SPA1 from the *SUC2* promoter in both conditions (Fig. II-15 C). Taken together, this suggests that SPA1 expressed in single tissues other than the epidermis cannot rescue the hypocotyl response to low R:FR conditions.

II.2 *SPA* genes interact with the auxin response

It has been recognised that the *spa* mutant phenotypes overlap with auxin-related phenotypes, such as short hypocotyls in darkness and light compared with the WT and a reduced number and size of leaf cells in true leaves (Ranjan et al., 2011; Fackendahl, PhD Thesis, 2011). Furthermore, the shade avoidance phenotype of *spa* triple and quadruple mutants also resembles an aberrant auxin response. Thus, the auxin response was analysed in seedlings in different light conditions and in adult leaves in WT and *spa* triple mutants. *DR5::GUS* was crossed with the *spa1 spa2 spa4* mutant (See Material and Methods for details on the selection of the mutants).

II.2.1 Auxin signalling in *SPA* triple mutant seedlings in darkness and low FR light

Seedlings of *DR5::GUS* and two lines from independent crosses of *spa1 spa3 spa4 DR5::GUS* and *spa1 spa2 spa4 DR5::GUS* were grown in darkness or low FR light (0.2 µmol $\times$ m^{-2} $\times$ sec^{-1}) for four days and a GUS staining was performed. Generally, all *DR5::GUS* lines employed throughout this study showed a pronounced staining at the root tip that reflects a local auxin signalling maximum, which was not observed to change in any condition. In a first set of experiments, high variations of the GUS signal distribution and intensity was observed within each genotype (data not shown). Nevertheless, the trend showed that the

GUS staining was strongest in dark-grown *DR5::GUS* seedlings, where the cotyledons and the apical hook were stained. Also in low intensities of FR light, the staining was weaker in DR5::GUS seedlings compared to dark-grown seedlings. The staining was overall weaker in the *spa1 spa3 spa4* and the *spa1 spa2 spa4* mutant backgrounds, exhibiting staining in dark-grown seedlings that resembles the Wc grown WT seedlings in case of the *spa1 spa2 spa4* seedlings.

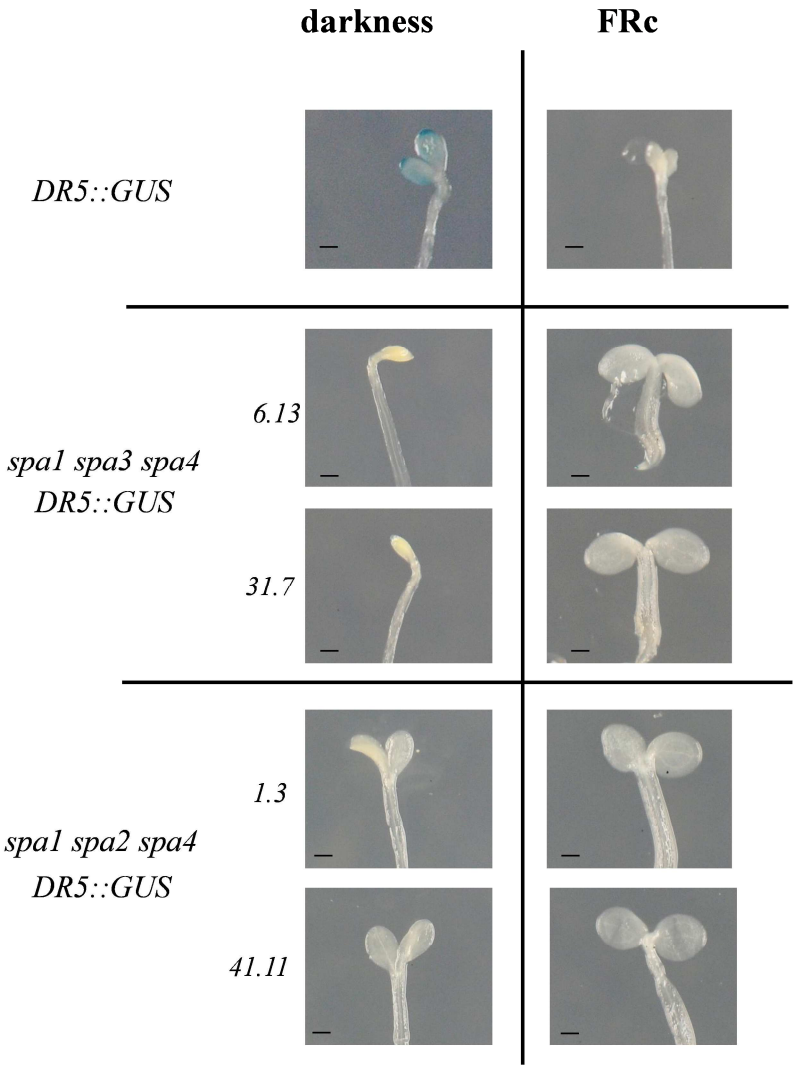

Figure II-16: The auxin response in darkness is reduced in the *spa1 spa3 spa4* and *spa1 spa2 spa4* mutants. GUS stainings of seedlings which were grown for four days in darkness or low continuous far-red light (FRc) (0.2 µmol $\times m^{-2} \times sec^{-1}$). The black bar represents 200 µm. The two lines per cross derived from independent crosses.

Subsequently, the experiment was conducted with an altered procedure that minimized manipulation of the seedlings prior to the GUS staining (Fankhauser, personal communication, 2011). In the second set of experiments with the new method, the staining was mostly weaker, but more reproducible results were obtained. GUS activity was visible in cotyledons of dark-grown DR5::GUS seedlings, but was almost absent from most of the cotyledons of FR grown seedlings even at low FR intensities (Fig. II-16). In most seedlings

from the *spa1 spa2 spa4* and *spa1 spa3 spa4* crossings, the GUS signal was faint or no staining was visible in the cotyledons in all conditions, apart from single seedlings that showed staining. This indicates that at the seedlings stage, the auxin response differs between WT and the *spa* triple mutants. Thus, the phenotypes of the *spa* mutant seedlings correlate with an altered auxin response, which could partly explain the shorter hypocotyl and photomorphogenesis of the mutants in darkness.

II.2.2 Auxin signalling is not enhanced by low R:FR in *spa1 spa3 spa4* and *spa1 spa2 spa4* mutants

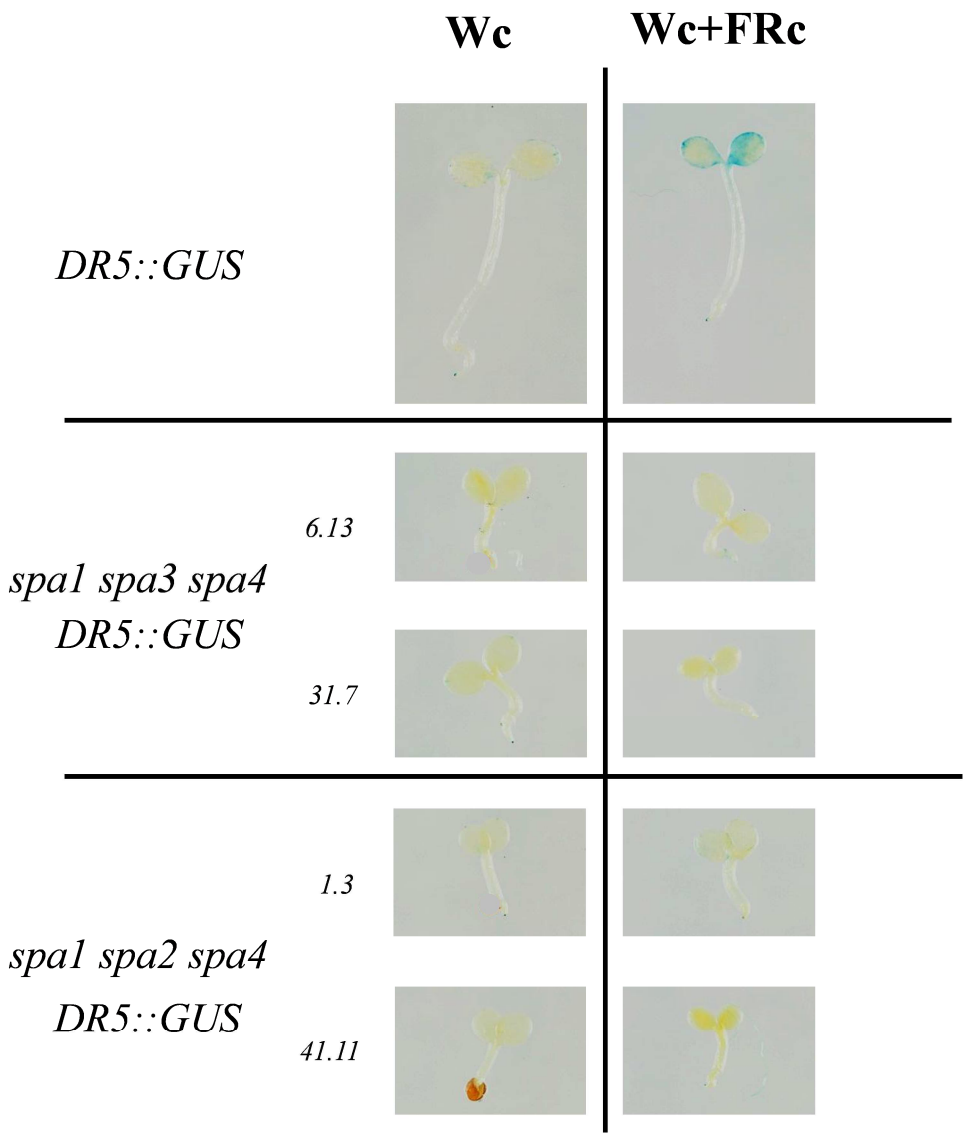

Figure II-17: *DR5::GUS* expression is not elevated in spa1 spa3 spa4 and spa1 spa2 spa4 mutants in response to low R:FR. GUS staining of seedlings grown for four days in Wc shifted to Wc+FRc for seven hours or kept in Wc (growth at 27°C). A representative seedling of *DR5::GUS* and of two independent crosses of *spa1 spa3 spa4 DR5::GUS* and *spa1 spa2 spa4 DR5::GUS* are shown.

DR5::GUS activity is enhanced in the cotyledons of seedlings by shade treatment, which indicates increased auxin signalling (Tao et al., 2008). Auxin is required for the hypocotyl elongation in response to low R:FR conditions (Tao et al., 2008; Keuskamp et al., 2010). Thus, the question was addressed, if auxin signalling may be affected in the cotyledons of *spa1 spa3 spa4* and *spa1 spa2 spa4* mutants that lack the elongation response of the hypocotyl and the petioles of the cotyledons to low R:FR conditions. Preliminary results with DR5::GUS lines in shade avoidance were obtained earlier under my supervision (Dickopf, Master Thesis, 2011). In order to enhance the faint *DR5::GUS* signals obtained in preliminary experiments, the seedlings were grown in 27°C, which causes overall elongation compared to growth at 21°C, but does not affect the elongation response to the low R:FR treatment (Figure S2). The majority of *DR5::GUS* seedlings showed a strong blue staining of the cotyledons in response to seven hours low R:FR treatment, while the Wc grown seedlings showed blue staining only at the margins of the cotyledons and in the root tip (Figure II-17). The pronounced increase in GUS staining in response to low R:FR was not observed in the *spa* triple mutant *DR5::GUS* lines tested. This indicates that the increase of the auxin response is absent from the two *spa* triple mutants that lack the elongation response of the hypocotyl in response to low R:FR and that *SPA* genes are involved in the enhancement of the auxin response in response to low R:FR conditions.

II.2.3 *SPA* genes regulate auxin biosynthesis genes in response to low R:FR

The reduced DR5::GUS expression in shade-treated *spa* mutants compared to WT may be caused by a lower auxin biosynthesis or reduced auxin signalling or altered auxin transport. *De novo* auxin biosynthesis by the TAA1 pathway is required for shade avoidance elongation responses (Tao et al., 2008). *TAA1* is thought not to be regulated by low R:FR conditions, but was shown to be a direct PIF4 target in temperature signalling (Tao et al., 2008; Franklin et al., 2011). *YUC* genes have also been implicated in the control of auxin biosynthesis in response to low R:FR and the expression of several *YUC* genes is responsive to low R:FR treatment in the hypocotyl and the petioles of true leaves (Konzuka et al., 2010; Tao et al., 2008; Won et al., 2011). Thus, the transcript levels of *YUC* genes in WT and the *spa1 spa3 spa4* triple mutant were measured from samples taken from 11-day-old plants (Fig. II-18A). *YUC2* transcript levels were neither elevated in the WT, nor in the *spa* triple mutant in response to the simulated shade. *YUC8* transcript levels were higher in response to low R:FR conditions in the WT, but the elevation by simulated shade conditions was diminished in the *spa1 spa3 spa4* mutant. The same trend was observed for *YUC9*, though the induction by low

R:FR conditions was not significant in the WT. This indicates that the transcript levels of *YUC8* are under the control of *SPA* genes in a shade-dependent manner. The *TAA1* transcript was determined in seedling tissue including WT, the *spa1 spa3 spa4* mutant and the *cop1-4* mutant grown in Wc and low R:FR conditions for the indicated time (Fig. II-18 B).

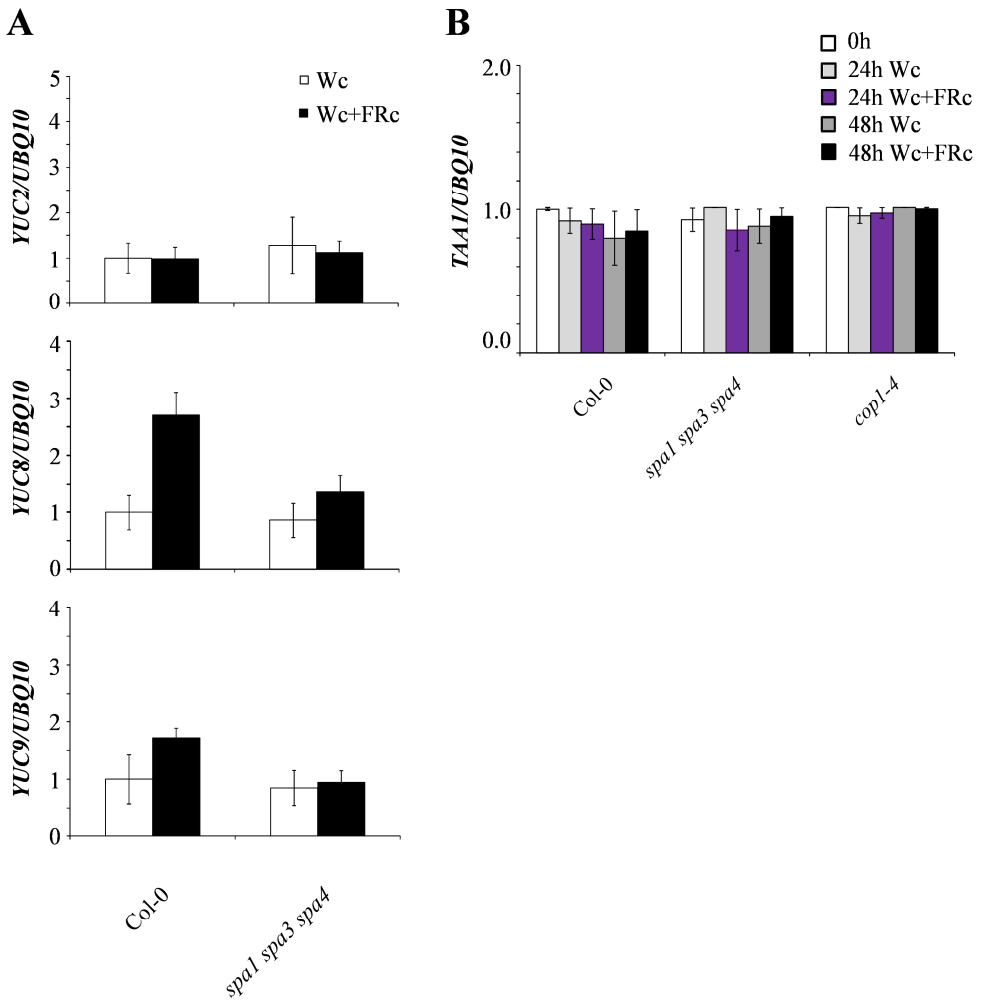

Figure II-18: Transcript levels of auxin biosynthesis genes in the *spa1 spa3 spa4* mutant in simulated shade.
A) Relative transcript levels of *YUC2, YUC8* and *YUC9* in Col-0 and *spa1 spa3 spa4* triple mutants under Wc and Wc+FRc were analysed in seedlings grown for 4 days under Wc and shifted to Wc+FRc for additional 7 days or kept in Wc. *UBQ10* was used as endogenous control. Data represent the mean of three biological replicates ± SEM. **B)** *TAA1* transcript levels in prolonged simulated shade (24h / 48h of Wc+FRc treatment compared to Wc treatment) in the *spa* triple mutant and *cop1-4* mutant. *UBQ10* was used as endogenous control. Data represent the mean of three biological replicates ± SEM.

The *TAA1* transcript levels were similar in all tested conditions and backgrounds. The transcript of *TAA1* was unresponsive to the low R:FR conditions after 24h or 48h of treatment compared to Wc in the WT the *spa* triple mutant and the *cop1-4* mutant. This suggests that *TAA1* is not differentially induced in our shade conditions and that *SPA* genes and *COP1* are not limiting for *TAA1* expression. Taken together, the data presented on auxin response in *spa* triple mutants and on the transcript abundance of *YUC* genes in the *spa* triple mutant backgrounds support the notion that *SPA* genes may act on the shade avoidance related

elongation responses in part by manipulating the auxin biosynthesis pathway. The transcript levels of *YUC* genes should also be measured at the seedling stage in order to correlate the hypocotyl and cotyledon phenotypes with the *YUC* transcript levels.

II.2.4 Auxin signalling in young leaves of adult *spa* mutants

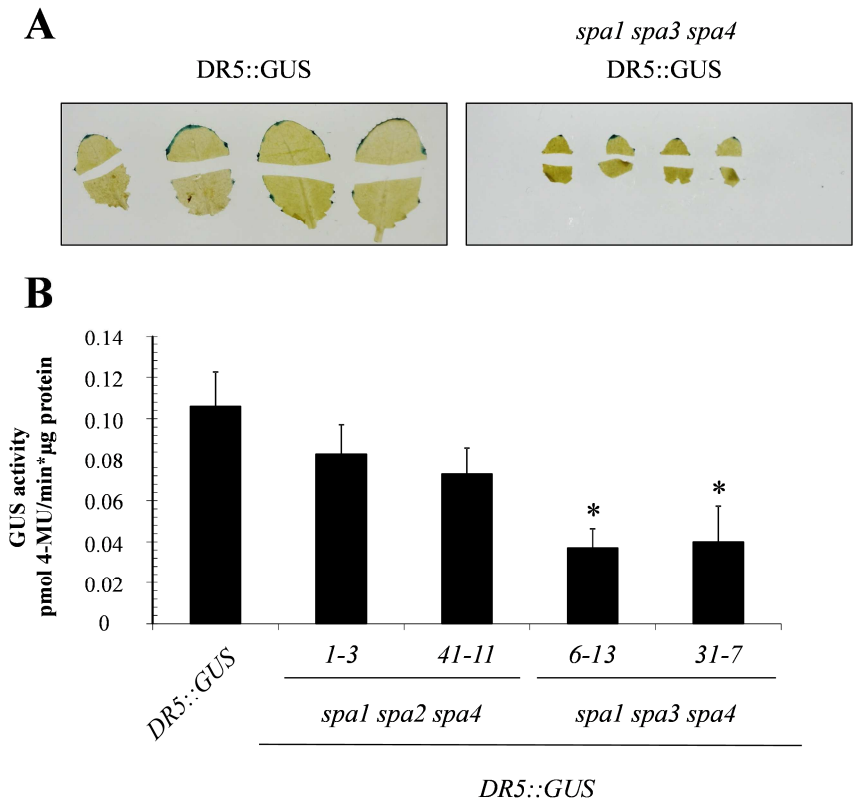

Figure II-19: Auxin response is reduced in young leaves of *spa1 spa3 spa4*. The auxin response in the youngest leaves of 2- week-old *DR5::GUS*, *spa1 spa3 spa4 DR5::GUS* and *spa1 spa2 spa4 DR5::GUS* plants, determined by GUS analyses. **A)** GUS staining of the youngest leaves of *DR5::GUS* and *spa1 spa3 spa4 DR5::GUS* plants. Leaves were bisected to allow uniform substrate uptake. **B)** A quantitative GUS analysis of *DR5* activity in Col-0, *spa1 spa2 spa4* and *spa1 spa3 spa4*. Samples were analysed in technical duplicates. Data are represented as the mean of three biological replicates ± SE. A student's t-test was performed and significant ($p<0.05$) differences compared with the WT background were asterisked(*).

The leaf size of *spa* mutants has been analysed previously and found to be reduced due to a lower cell number and a diminished cell size when compared to the WT (Fackendahl PhD Thesis, 2011). SPA4 is the main *SPA* gene acting on adult plant growth (Fackendahl, PhD Thesis, 2011). *SPA1* can act non-cell-autonomously from the vascular tissue or the mesophyll to control the leaf size, which indicates that they may act on hormone pathways (Ranjan et al., 2011). Thus, the question was addressed, if auxin response was altered in the young leaves of *spa1 spa3 spa4* (that grows small leaves compared to the WT, Fig. II-19 A) and the *spa1 spa2 spa4* mutant that grows larger leaves compared with the *spa1 spa3 spa4* triple mutant more similar to the WT.

The activity of the *DR5* promoter was analysed in the youngest leaves (where expansion takes place) of two-week-old plants grown in long days (LD) (Fig. II-19). The GUS staining was strong along the leaf margins in the WT background, but was confined to the leaf tip in the *spa1 spa3 spa4* mutant (Fig. II-19 A). The lack of auxin response alongside most of the leaf margins correlates with the dwarfed leaf phenotype of this mutant, because elevated auxin response is associated with cell elongation and division in growing tissues. The auxin response in the leaves was also quantified with a fluorometric MUG assay (Fig. II-19 B). The WT and the *spa1 spa2 spa4* mutant exhibited higher *DR5::GUS* activities compared to the *spa1 spa3 spa4* triple mutant in the youngest leaves of two-week-old plants. This correlates with the phenotypes, as *spa1 spa2 spa4* mutants grow larger leaves than *spa1 spa3 spa4* mutants (Fackendahl PhD Thesis, 2011) and suggests that reduced auxin response may be involved in the dwarfed *spa* mutant phenotypes.

II.2.5 Auxin-responsiveness of seedlings in darkness and light

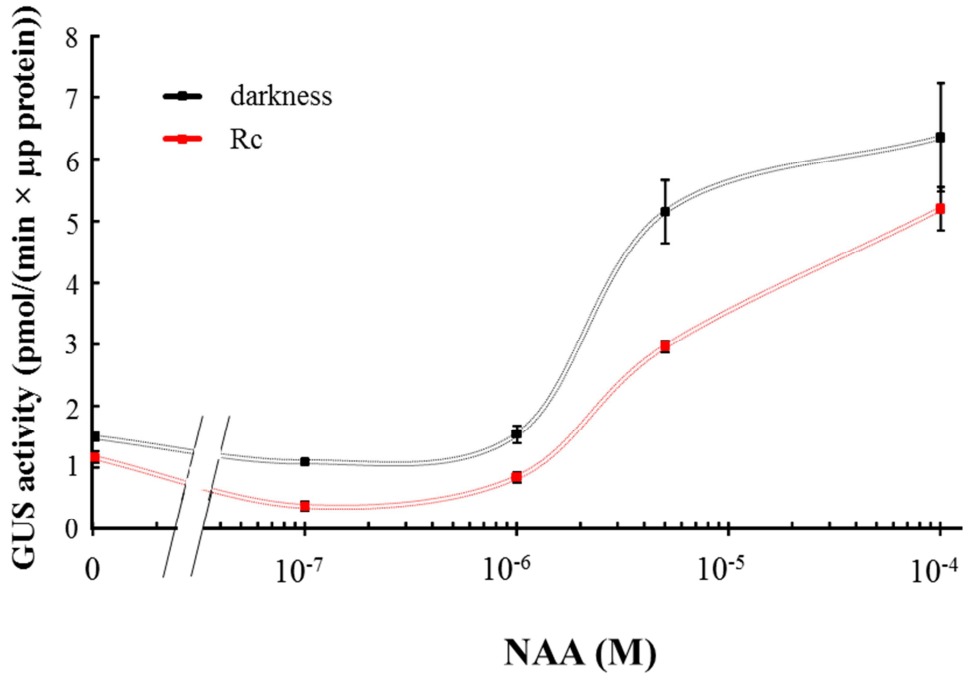

Figure II-20: 1-naphthaleneacetic acid (NAA) dose-response curve of DR5::GUS seedlings in darkness and light. Seedlings were grown in darkness for five days in liquid culture and treated with different NAA concentrations or mock-treated. Seedlings were then incubated in darkness or light for 24 hours. A quantitative GUS assay was performed. The data represent the mean of three biological replicates ± SEM.

If light affects auxin response on the level of auxin-responsiveness of the tissues, the auxin-responsiveness to externally applied auxin should differ between light and darkness. In order to address this hypothesis, a series of dose-response curves with increasing 1-

naphthaleneacetic acid (NAA) concentrations were conducted. *DR5::GUS* seedlings were grown in darkness and transferred to continuous white light or kept in darkness in liquid cultures and supplemented with different concentration of NAA for 24 hours. A difference in the response was repeatedly observed in the fluorometric assays most notably between 10^{-7} and 10^{-5} M NAA, as light-grown seedlings showed a lower response in this range, but similar responses at higher concentrations (data not shown). This was also observed in the representative transfer experiment shown in figure II-20. The induction of *DR5::GUS* activity is increasing with a different slope between 10^{-6} and 10^{-5} M externally applied NAA.
Taken together the data favour a slight light-dependent difference in auxin-responsiveness of seedlings, pointing towards a direct light-mediated manipulation of auxin signalling components.

II.2.6 *COP1* and *SPA* act on root elongation in darkness in an auxin-transport dependent manner

It has been observed previously that *cop1* mutant plants kept developing under dark conditions and even flowered when supplied with sucrose (McNellis et al., 1994; Ranjan, A., unpublished data). In contrast to WT seedlings that arrest root growth in darkness at some point roots were found to further extend in prolonged darkness in the *cop1-4* background. Root elongation is under the control of multiple phytohormones, including auxin, which is necessary for root initiation and root growth, but can also inhibit primary root growth depending at high auxin levels in the tissue (Rahman et al., 2007). Thus, it was tested for the auxin dependency of the root elongation phenotype in the *cop1-4* mutant. The root length of 11- and 15-day-old dark-grown *DR5::GUS*, the constitutive photomorphogenic *spa1 spa2 spa4 DR5::GUS* and *cop1-4* seedlings, was determined on MS with 1% sucrose and with or without auxin transport inhibitor 1-N-naphthylphtalamic acid (NPA) (Fig. II-21 A-C). The WT arrested root growth as it has been reported and exhibited similar root lengths on plates with and without NPA. Both, the *spa1 spa2 spa4 DR5::GUS* mutant lines and the *cop1-4* mutant displayed longer roots on MS plates compared to the WT after 11 days that were even longer after 15 days, indicating further extension beyond day 11 in these backgrounds. The exaggerated growth of the roots in the two mutant backgrounds was reversed on NPA.

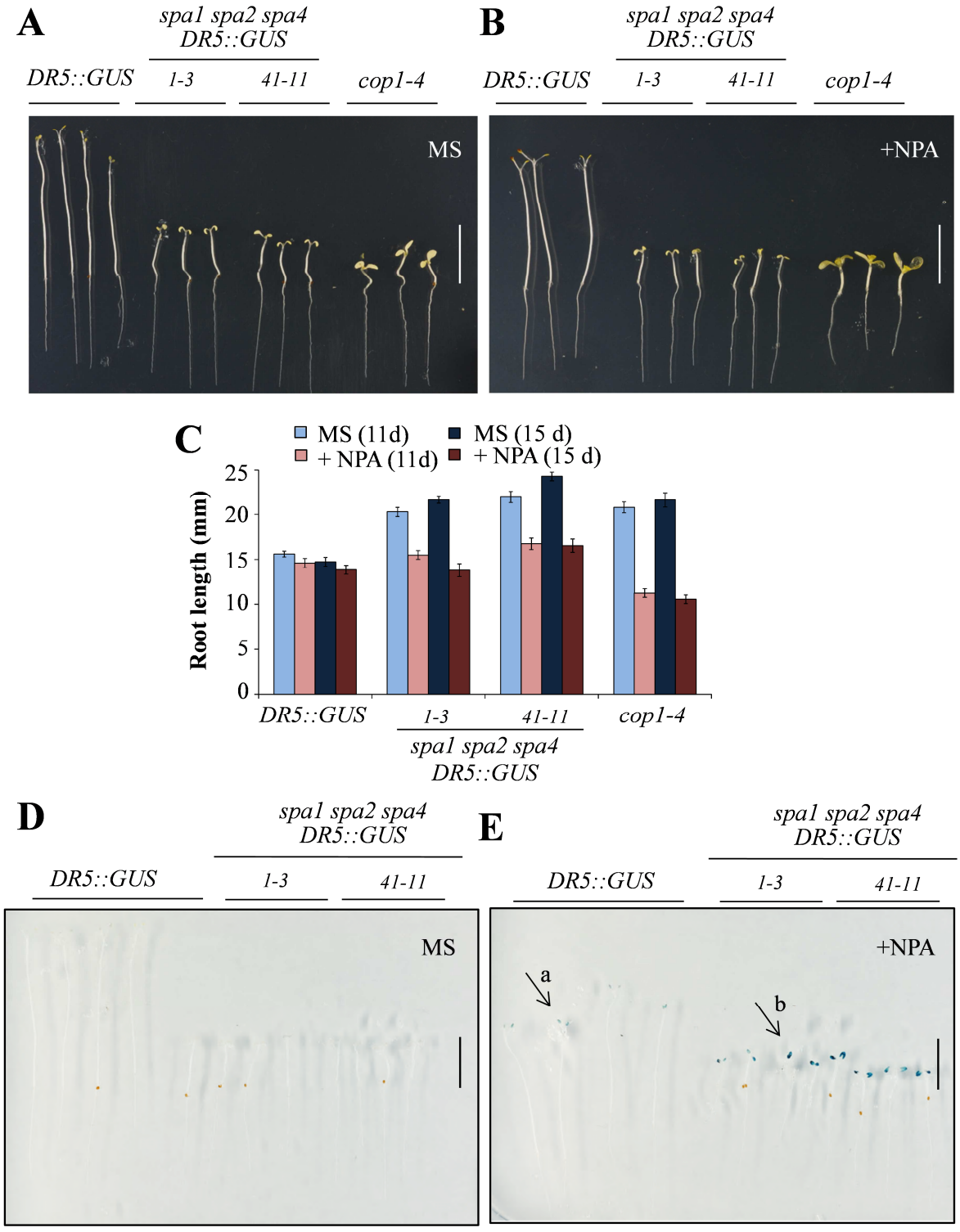

Figure II-21: Roots of *spa1 spa2 spa4* and *cop1-4* show elevated elongation in darkness that is reversed by NPA.
A+B) Phenotype of 15-day-old seedlings grown in darkness on vertical MS + 1% sucrose plates **(A)** or plates containing in addition 5 µM NPA **(B).** The white bar represents 10 mm. **C)** Root length of *DR5::GUS*, *spa1 spa2 spa4 DR5::GUS* lines and *cop1-4* mutant after 11 and 15 days of growth in darkness on vertical MS +1% sucrose plates or additionally on 5 µM NPA. Data shown as the mean of measured roots ± SEM (n ≥ 20). **D+E)** GUS staining of 15-day-old *DR5::GUS* and *spa1 spa2 spa4 DR5::GUS* seedlings grown in darkness without **(D)** or with **(E)** 5 µM NPA. Representative seedlings are shown. The black bar represents 10 mm. The arrows indicate the position of a cotyledon of **a)** *DR5::GUS* and **b)** *spa1 spa2 spa4 DR5::GUS* seedlings.

This suggests an essential requirement for auxin transport of the observed elongation of the roots in *spa1 spa2 spa4* and *cop1-4* mutants, while normal root elongation in darkness in the WT was independent of auxin transport.

A subset of the 15-day-old *DR5::GUS* and *spa1 spa3 spa4 DR5::GUS* seedlings were analysed with a GUS staining to analyse the intensity of auxin signalling in the WT and the mutants (Fig. II-21 D,E). In the seedlings grown on MS plates, GUS staining was absent from the cotyledons (Fig. II-21 D). The cotyledons of *DR5::GUS* seedlings grown on MS supplemented with NPA, exhibited blue staining (Fig. II-21 E). The staining was much more intensive in the *spa1 spa2 spa4* seedlings tested. This indicates that the auxin response increased in the cotyledons when polar auxin transport was blocked and that the auxin response was stronger in *spa1 spa2 spa4* mutants in darkness when auxin transport was blocked. These data point towards increased auxin levels in the *spa1 spa2 spa4* mutant in darkness or altered responsiveness of auxin in the cotyledons.

To determine the NPA dependency of root growth in the light in WT and *spa* and *cop1* mutant, dark-grown seedlings were compared with Wc-grown seedlings in a second set of experiments. Roots of 15-day-old *spa1 spa2 spa4* mutants and *cop1-4* were shorter in Wc compared to the WT, but longer than in the respective dark-grown seedlings. NPA had an effect on the root length of the WT and the mutants in Wc, but only on the root length of *spa1 spa2 spa4* mutants and the *cop1-4* mutant in darkness (Fig. II-22).

This suggests that NPA reversed the exaggerated root extension in the mutants in darkness, but NPA does not contribute to the shorter root of *spa* triple mutant and *cop1-4* in Wc. This leads to the speculation that *SPA* genes and *COP1* may regulate auxin transport or are involved in the root length control in an auxin-independent process in darkness.

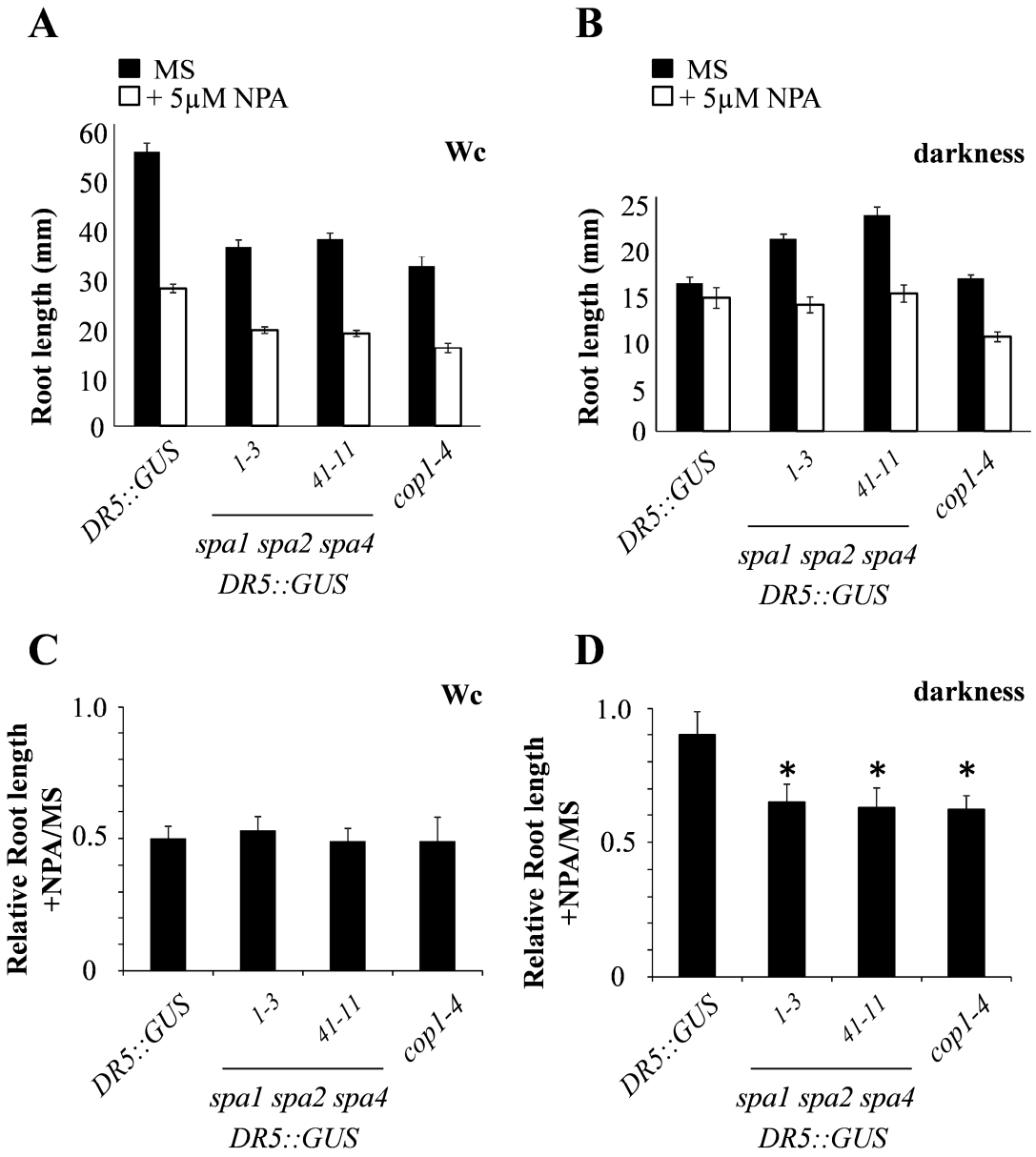

Figure II-22: The root length is decreased by NPA in the WT only in Wc, but in Wc and darkness in *spa* triple mutant and *cop1-4*. Seedlings were grown in white light (Wc) or darkness for 15 days on vertical MS plates containing 1% Sucrose +/- auxin transport inhibitor (5 µM NPA). **A+B)** Total root length measurments of Wc or dark-grown seedlings of WT, *spa1 spa2 spa4 DR5::GUS* and *cop1-4*. **C+D)** Relative root length in Wc and darkness comparing growth on plates with and without NPA calculated as ratio. The mean of the root length on MS+NPA was divided by the mean on MS and the data are presented as mean ± SEM. A student‘s t-test was performed and significant ($p<0.05$) differences from the WT were asterisked(*).

II.3 Functional promoter analysis of light and auxin regulated genes

At the onset of photomorphogenesis, a high number of genes are swiftly regulated, most of which are up-regulated to serve functions in the light growth of the seedling. Other genes are down-regulated in response to light. This down-regulation may originate from a loss of dark-up-regulation or a repression by a light-activated factor or additional indirect effects (Tepperman et al., 2006). Among the down-regulated genes, known hormone responsive genes were found to be over-represented, such as genes responsive to auxin. These genes include *IAA19*, *IAA29* and *SAUR-AC1/SAUR15* and several *SAUR-like* genes, such as *SAUR-AC1-l* (At4g13790). This led to the question, if the light signal has a direct impact on the regulation of these genes. In order to unravel the interactions of light and auxin signalling pathways in regulation of the expression of auxin up- and light down-regulated genes, an analysis of the regulation was performed on the level of the promoters. It was hypothesized that, if auxin was to solely account for light repression, AuxREs should be essential for the light regulation of the genes.

SAUR-AC1-l, a member of the *SAUR-LIKE* gene family contains a transcribed 509 base pair (bp) fragment, which contains a single 279 bp exon encoding a 92 amino acid (aa) protein (11 kda protein). The 5' promoter region up to the next gene is 2074 bp long including the 5' untranslated region (UTR) and was included in the analysis in this study.

First, 2.5kb fragments (or in case of *SAUR-AC1-l* the 5' region up to the neighbouring gene) of the 5' untranslated regions of candidate genes were fused to luciferase (*LUC*) and β-glucuronidase (*GUS*) genes and stably transformed into Arabidopsis. The down-regulation of promoter activity by light was more prominent in the lines expressing the luciferase, presumably due to a higher GUS protein stability (Koo et al., 2007, data not shown). Thus, the analysis was continued with the lines expressing LUC.

Seedlings of independent T2 lines were grown in darkness for three days and subsequently shifted to Rc (30 µmol × m^{-2} × sec^{-1}) or kept in darkness. The LUC activity per µg total protein was determined for each individual line and condition and the ratio of LUC activity in Rc and darkness was calculated. The mean values of the ratio of the LUC activity in Rc and darkness of the *promoter::LUC* expressing lines are presented in figure II-23 (results of individual T2 lines: Supplement figure S3).

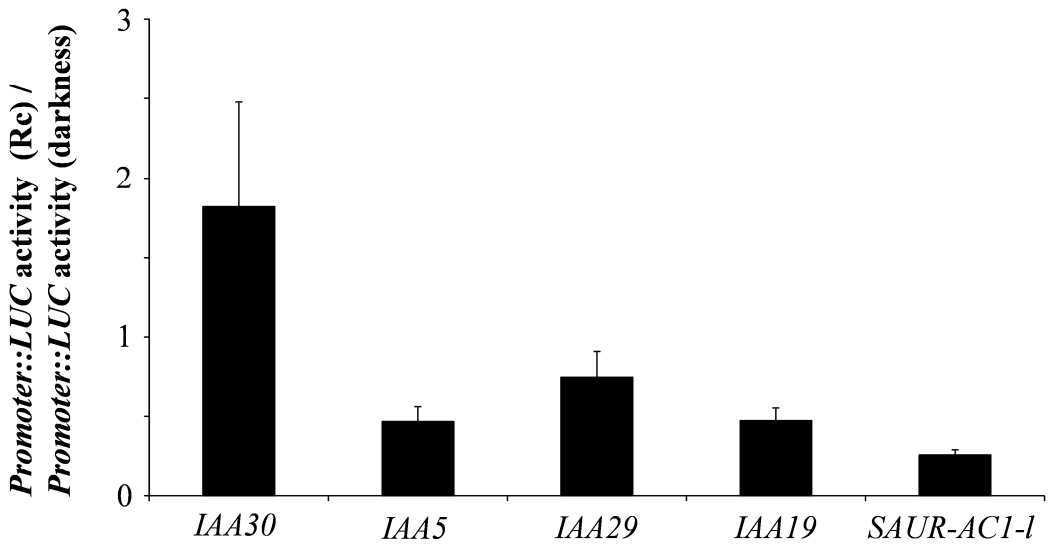

Figure II-23: Light regulation of auxin-induced promoters. A set of quantitative luciferase analyses (qLUC) was performed with *Promoter::LUC* lines of auxin-responsive genes (T2). Seedlings were grown in darkness for four days and transferred to Rc (30 µmol × m^{-2} × sec^{-1}) for 24 h or kept in darkness. Around 50 mg of tissue was harvested and luciferase activity measurements and protein estimation performed with protein extracts in technical duplicates. The ratio of LUC activity (counts (photons)/(10 sec × µg protein)) in Rc divided by the activity in darkness was calculated for each individual line. The mean of ≥ 15 T2 lines is presented with error bars indicating ± SEM.

The two *Aux/IAA* promoters, *IAA5* and *IAA30*, were also analysed, though the genes were previously not reported to be pronouncedly regulated by light on the transcript level (Tepperman et al., 2001 and 2006). While the luciferase activity of the T2 lines expressing LUC from the *IAA30* promoter showed a slight upregulation of the expression in red light in most lines compared to darkness (Fig. II-23 and Supplemental Fig S3), the *IAA5::LUC* lines showed a down-regulation by Rc. The *IAA29* promoter caused a decrease of the luciferase signal in the light compared with dark-grown seedlings, but an overall weak luciferase activity in the T2 lines (Fig. II-23, Fig. S3).

The two constructs *IAA19::LUC* and *SAUR-AC1-l::LUC* showed a strong luciferase activity in darkness and Rc. Furthermore they both exhibited a significant repression of the luciferase activity by red light, which is present in most lines tested (Fig. S4).

The two promoters, *IAA19* and *SAUR-AC1-l* that showed the highest overall expression and a clear light regulation, were chosen for a deeper analysis of auxin and light regulation. Also, *IAA19* has been proposed to be a good candidate for a directly light-regulated *Aux/IAA* (Sibout et al., 2006). In order to confirm the auxin induction and light reduction of the transcript levels of *IAA19* and S*AUR AC1-l*, a qRT-PCR was performed prior to the promoter function analysis (Fig. II-24).

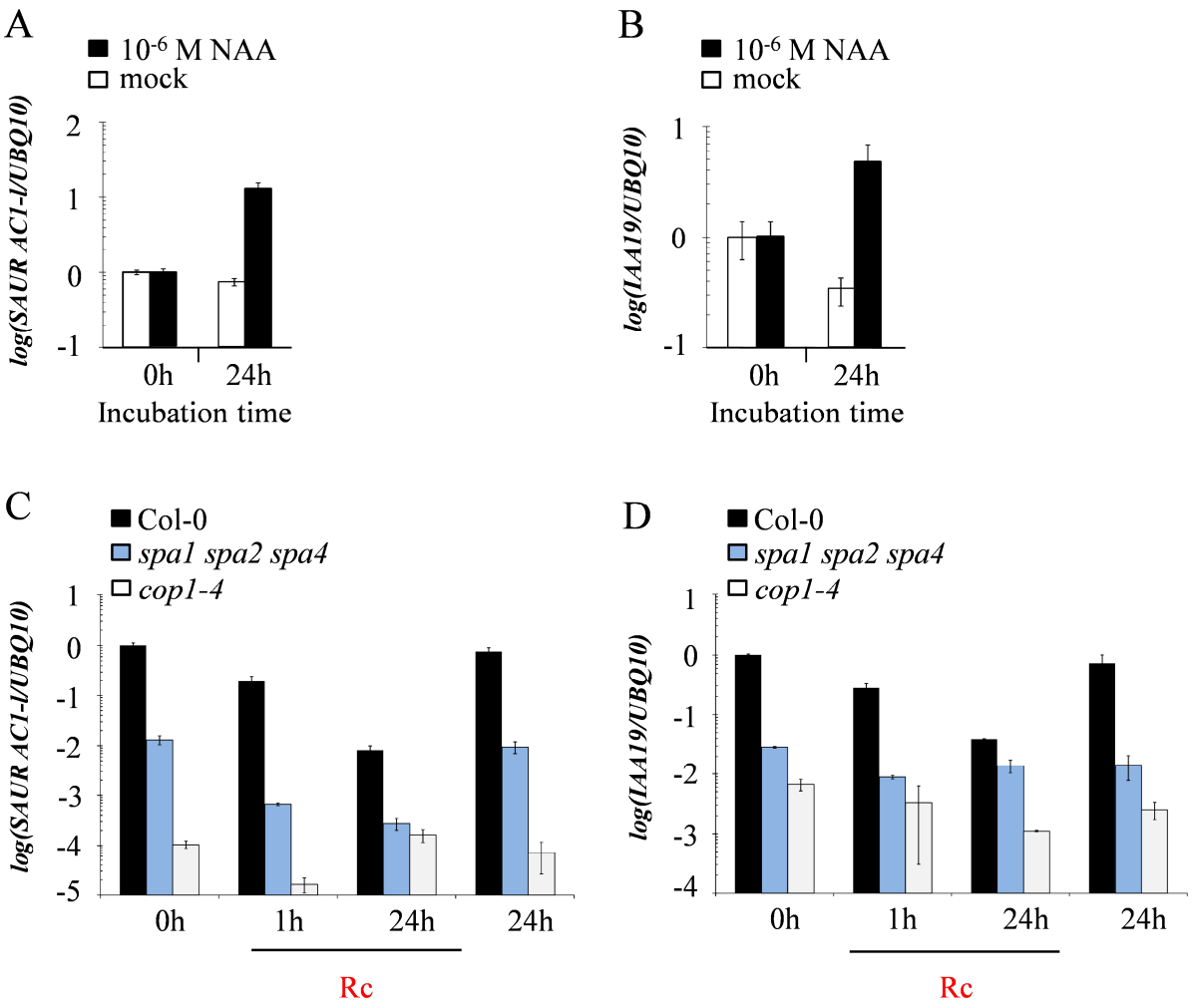

Figure II-24: Auxin inducibility and light repression of *IAA19* and *SAUR-AC1-L* transcript levels. Transcript levels were analysed using qRT-PCR. *UBQ10* was used as endogenous control and data were calibrated to 0 hours (Col-0). The data are presented as mean of two biological replicates ± SEM on a logarithmic scale. **A+B)** Col-0 seedlings were grown in liquid MS + 1% Sucrose in continuous white light (Wc) and treated with 10^{-6} M NAA or mock-treated for 24 hours and qRT-PCRs performed. **C+D)** Four day-old dark-grown Col-0, *spa1 spa2 spa4* and *cop1-4* mutant seedlings were incubated in continuous red light (Rc; 30 µmol × m^{-2} × sec^{-1}) for the indicated time or kept in darkness and qRT-PCRs performed.

Both transcripts accumulated in the samples treated for 24 hours with NAA compared to the mock-treated samples (Figure II-24 A,B). In the same way, the transcripts were down-regulated by continuous red light treatment over the course of 24 hours with a substantial reduction observed after one hour. These results are in agreement with the auxin-inducibility and light regulation of the genes previously reported in microarray studies (see also: Goda et al., the AtGenExpress Consortium, http://jsp.weigelworld.org/expviz/expviz.jsp.), supporting the view that the two genes are good candidates to be studied further.

Similarly to what can be observed for virtually all light up-regulated genes (e.g. *Chlorophyll a,b-binding protein* (*CAB*)) both light down-regulated genes showed lower transcript levels in the *spa* triple mutant background and *cop1-4* in dark conditions (Fig. II-24 C,D) as the

transcriptomes of dark-grown *cop1-4* largely resembles the one of light-grown WT seedlings (Ma et al., 2002).

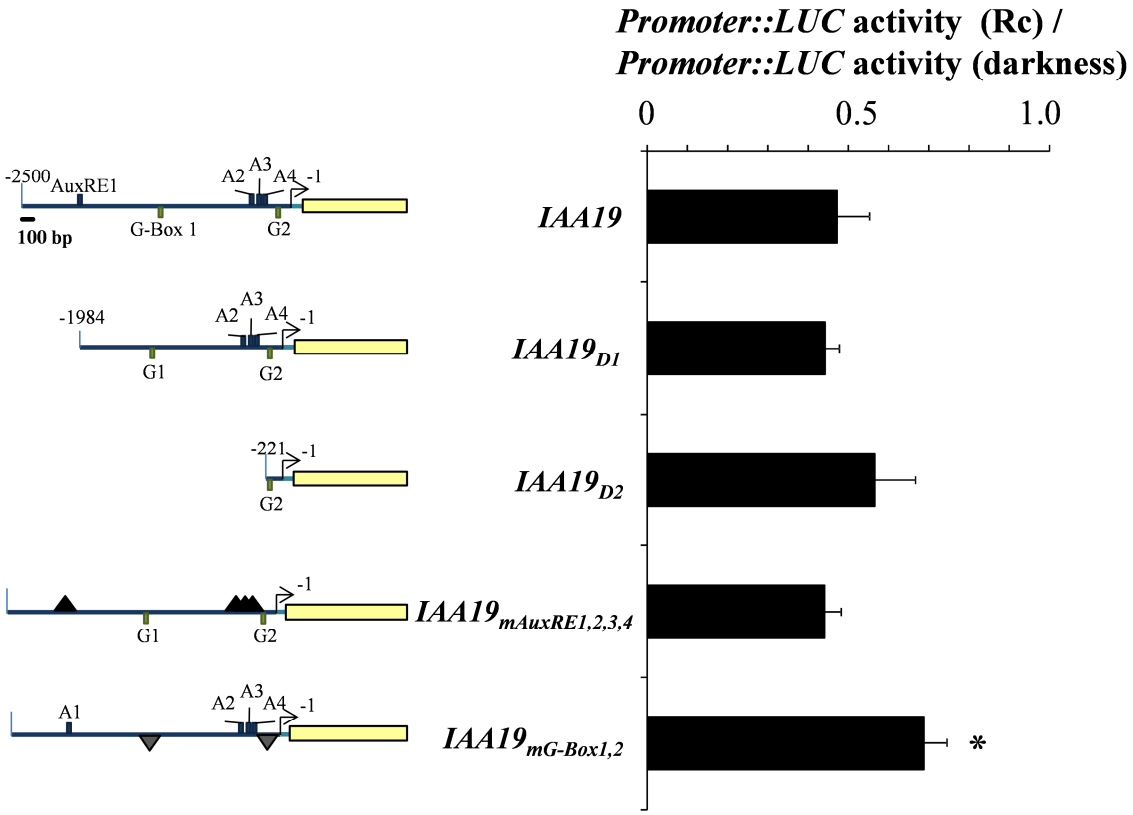

Figure II-25: Activity of *IAA19* promoter deletion constructs and point-mutated constructs in darkness and light. The 5‘ 2.5 kb promoter fragment and mutated and deleted versions of *IAA19* were cloned and fused to the firefly luciferase (LUC, yellow boxes). The dark blue boxes indicate the position of the four canonical AuxRE core motifs (A1-4), the green boxes represent the two G-Box motifs (G1-2). Triangles represent mutated motifs. A set of quantitative luciferase analyses (qLUC) was performed with individual lines of the indicated *IAA19::LUC* constructs (T2). Seedlings were grown in darkness for four days and transferred to continuous red light (Rc; 30 µmol × m^{-2} × sec^{-1}) for 24 h or kept in darkness. Around 50 mg of tissue was harvested and luciferase activity measurements and protein estimation performed with protein extracts in technical duplicates. The ratio of LUC activity (counts (photons)/(10 sec × µg protein)) in Rc divided by the activity in darkness was calculated for each individual line. The mean of ≥ 18 T2 lines is presented ± SEM (exception: I*AA19D2*: seven T2 lines).

In addition to AuxRE core motifs (TGTCTC) that were present in both promoters, core motifs of G-Box elements (CACGTG) that represent well-characterised LREs bound by PIF and other light signalling factors, such as HY5, were detected in the sequence of the two promoters. These core motifs were subsequently checked for their contribution to the light and auxin regulation of the genes. To this end, mutated versions of the *IAA19::LUC* promoter-reporter construct were generated by applying a site-directed PCR approach. The mutated sites were the four *AuxRE* elements core-motifs TGTCTC that were altered from TGTCTC to TGgCTC (mAuxRE). The introduced mutation was reported to abolish ARF binding to the sequence in the *DORNRÖSCHEN* (DRN) promoter causing the inhibition of

auxin induction of the corresponding gene and has already been known from early studies of AuxREs (Cole et al., 2009; Ulmasov et al., 1997a). Additionally, the two G-Box elements were also mutated by two point mutations in the decisive central nucleotides from CACGTG to CA**at**TG (mG-Box) (Izawa et al., 1994). I also generated deletion constructs of the 2.5 kb *IAA19* promoter named deletion construct one (*D1*) and deletion construct two (*D2*) (Fig. II-25). The first deletion construct contains three AuxRE elements and both G-Boxes, while the second fragment only contains the second G-Box and contains no AuxRE core motif. Transgenic plants were generated and processed as described for the *IAA19::LUC* construct, the resulting T2 lines screened for luciferase activity and mean values of the Rc to darkness ratio of the LUC activities calculated (Fig. II-25). The individual results for each line are presented in Figure S5. The *IAA19*$_{D1}$*::LUC* lines showed a similar Rc/d ratio of LUC activity compared to the full-length fragment analysed previously. The *IAA19*$_{D2}$*::LUC* lines exhibited very low luciferase signal intensities under both conditions in all lines examines (seven), which likely reflects the loss of important regulatory elements that uphold overall transcription, though the core promoter is expected to be included in the chosen region (Supplemental fig…). The mean value of the repression of LUC signal by light was significantly higher for the *IAA19*$_{mG\text{-}Box1,2}$*::LUC* lines compared to the IAA19*D1::LUC*, the IAA19*mAuxRE1,2,3,4::LUC* and also the *IAA19::LUC* lines, but was still detectable in most T2 lines which is also represented by the mean value (Fig. II-25 and fig. S4).

Taken together, the data suggest a function for the two deleted G-Box core motifs in the light regulated repression of *IAA19*. The deleted AuxRE core motifs on the other hand did not influence regulation of *IAA19* in Rc in this study.

Mutated versions of the *SAUR-AC1-l* promoter were generated as described above for the *IAA19* promoter. One core motif was found for each, AuxRE and G-Box, respectively. Also, two deletion constructs were generated that covered only the G-Box or none of the two elements (Fig. II-26). The first deletion construct (*D1*) resulted in comparable LUC activities and repression of activity after transfer to Rc compared to the full length construct (Fig. II-26, Fig. S5), but the second deletion construct (*D2*) showed a reduced regulation in response to light and a reduced overall activity in both conditions compared to the *SAUR-AC1-l* full length and the *D1* construct. In lines that contain the mAuxRE (*mAuxRE* and *mAuxRE mG-box*), the repression by light was considerably released, while lines carrying the *SAUR*$_{mG\text{-}Box}$*::LUC* construct still showed a strong decrease of luciferase activity between dark and light-grown seedlings. Taken together, in contrast to *IAA19*, where *G-Box* elements were

more important for light regulation of the promoter than the AuxREs, a function in the light regulation could be assigned to the mutated AuxRE, but not to the G-Box motif.

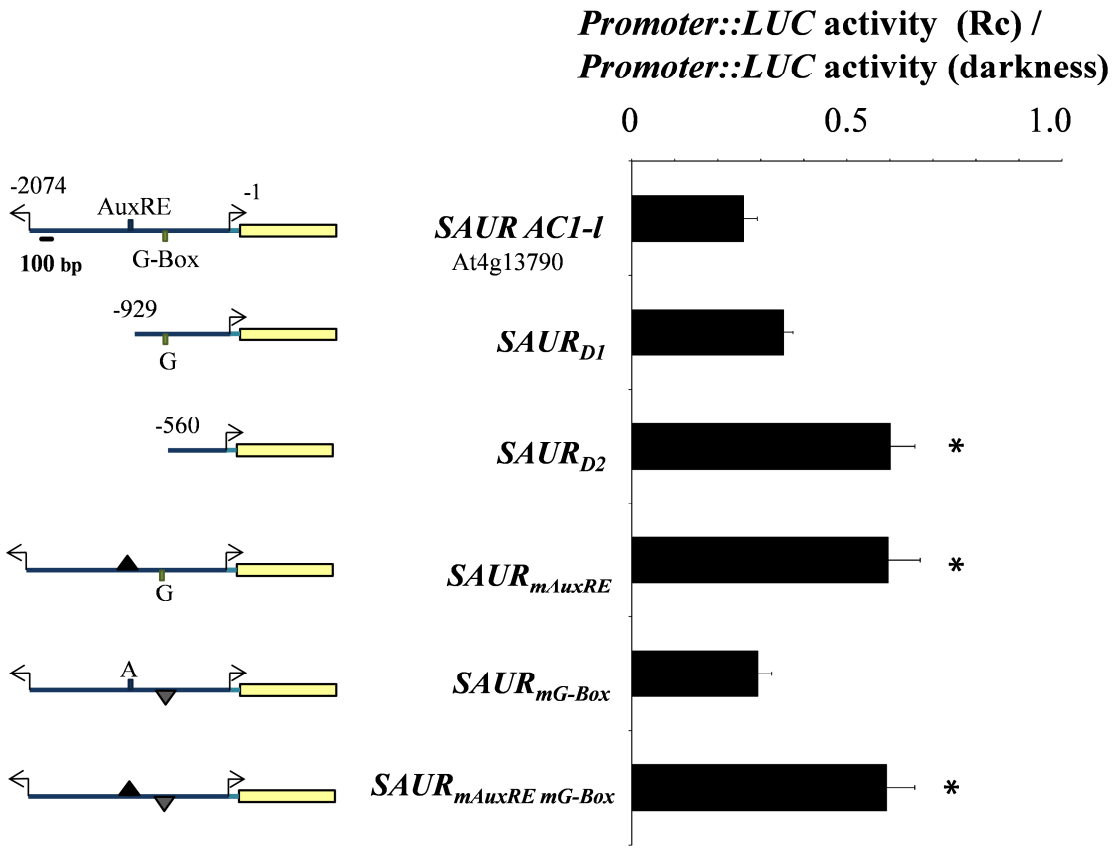

Figure II-26: Activity of *SAUR-AC1-l* promoter deletion constructs and point-mutated constructs in darkness and light. The *SAUR-AC1-l* genomic region and the mutated and deleted versions were cloned and fused to the firefly luciferase (LUC, yellow boxes). The dark blue box indicates the position of the canonical AuxRE core motif (A), the green box represent the G-Box motif (B). The triangles represent mutated versions of the motifs. A set of quantitative luciferase analyses (qLUC) was performed with individual lines of the *SAUR::LUC* constructs (T2). Seedlings were grown in darkness for four days and transferred to Rc (30 µmol × m^{-2} × sec^{-1}) for 24 h or kept in darkness. Around 50 mg of tissue was harvested and luciferase activity measurements and protein estimation performed with protein extracts in technical duplicates. The ratio of LUC activity (counts (photons)/(10 sec × µg protein)) in Rc divided by the activity in darkness was calculated for each individual line. The mean of ≥16 T2 lines is presented ± SEM.

On the other hand, the $SAUR_{D1}$ fragment that does not contain the AuxRE is still responsive to the Rc treatment, while the $SAUR_{D2}$ fragment that lacks both core motifs shows a diminished response. It is hypothesised that the SAUR full length promoter may contain additional sites in the region missing in the SAURD1 fragment that confer negative influence on the light regulation, which is relieved in the *D1* fragment and counteracted by the AuxRE.

The auxin response curve of *IAA19* determined from seedlings grown in liquid culture, exhibited a steeper slope than the slope observed with the $IAA19_{mAuxRE1,2,3,4}$ lines (Fig. II-27). This indicates that the auxin response was diminished in these lines by the introduced mutations. This was also observed in a preliminary experiment, where representative lines of *IAA19D1*, *IAA19D2*, *IAA19mG-Box1,2* and *IAA19mAuxRE1,2,3,4* were treated with 10^{-6} M

NAA or mock treated and the fold induction was compared between the lines. While the other lines all showed an induction between 10- and 100-fold, the three mAuxRE lines exhibited a 10-fold or less induction of the LUC signal (data not shown).

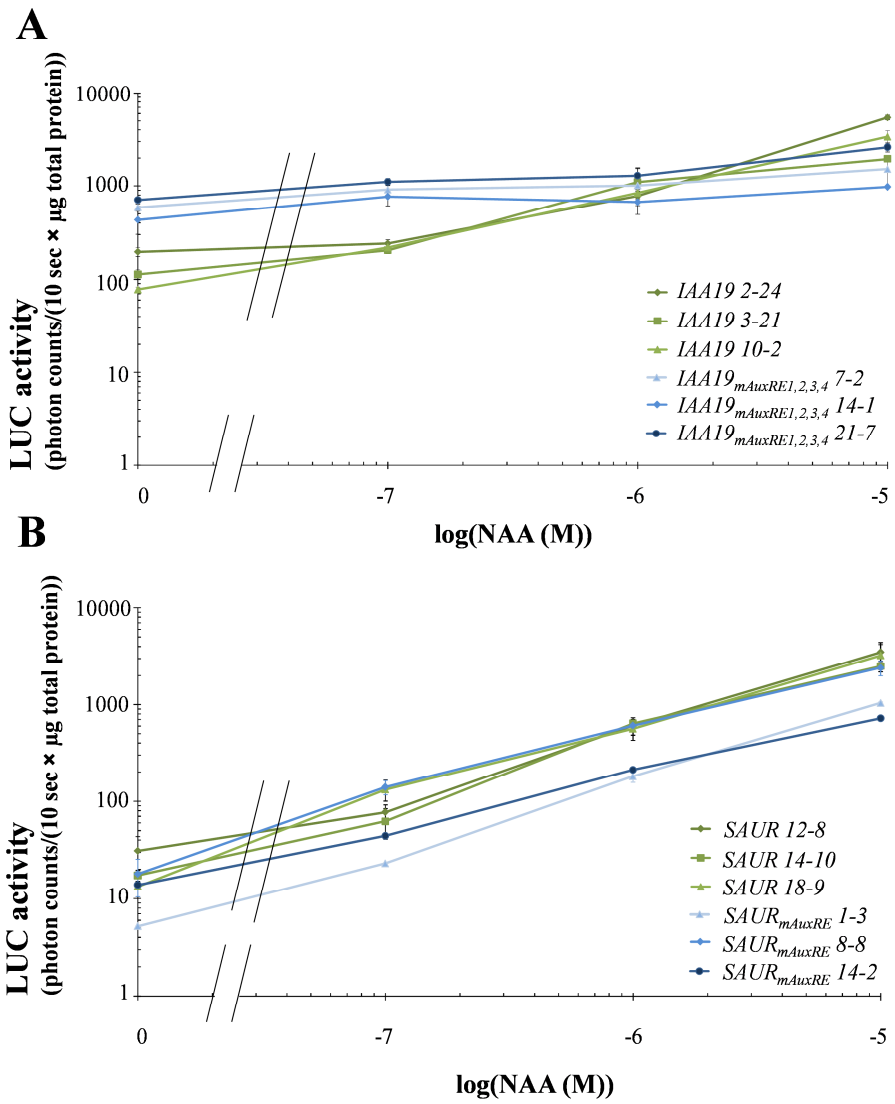

Figure II-27: Auxin dose-response of *IAA19*, *IAA19*$_{mAuxRE1,2,3,4}$, *SAUR (AC1-L)* and *SAUR*$_{mAuxRE}$ *promoter::LUC* lines. The luciferase activity was analysed in protein extracts from liquid culture grown seedlings treated with distinct NAA concentrations for 24h or mock-treated (logarithmic scales). Data represent the mean of three biological replicates ± SEM. **A)** Auxin response curves of three transgenic *IAA19::LUC* and *IAA19*$_{mAuxRE1,2,3,4}$ *::LUC* lines (T4). **B)** Auxin response curves of three transgenic *SAUR (AC1-L)::LUC* and *SAUR*$_{mAuxRE}$ *::LUC* lines (T4).

The auxin inducibility of the promoter constructs was tested subsequently in an NAA-induction assay based on liquid MS grown seedlings (Fig. II-27).

SAUR-AC1-l lines showed a similar slope with increasing NAA concentrations, indicating that they respond to NAA in the same way, suggesting that the mutation does not alter auxin responsibility of the promoter in the range of this experiment (Fig. II-27B). In a preliminary set of experiments, similar results were obtained for all *SAUR AC1-l* constructs, further

supporting the notion that *promoter::LUC* lines carrying the *mAuxRE* elements are still responsive to auxin (data not shown).
Taken together, the *AuxRE* mutated in the *SAUR AC1-l* promoter that showed a function in the light regulation of *SAUR AC1-l* did not alter the inducibility of the construct by exogenous NAA. Reversely, the four mutated AuxRE core motifs of the *IAA19* promoter were not required for normal light regulation of the $IAA19_{mAuxRE1,2,3,4}$*::LUC* constructs, but evidence points towards an involvement of the AuxRE in the NAA responsiveness of the promoter.

It was repeatedly attempted to also analyse the light regulation of all promoter constructs of *IAA19* and *SAUR* together in one experiment in the T3 and T4 generation in homozygous lines, but the results were not as convincing as the T2 data and not repeatedly reproducible. This might be due to the handling of the high number of LUC samples that are worked with during this experiment, as experiments with lower sample number resulted in reproducible results, such as the NAA treatment and the shade avoidance analysis.

However, here, evidence is provided that suggests two distinct mechanisms by which auxin-responsive genes may be repressed at the onset of photomorphogenesis. *AuxREs* may play an important role in the expression of some genes (e.g. *SAUR AC1-l*), while direct light signalling via *G-Box* elements could provide a more direct repression of other genes (e.g. *IAA19*) that may modulates auxin-responsiveness by light.

III. Discussion

III.1 *SPA* gene function in the shade avoidance syndrome of *Arabidopsis thaliana*

Shade avoidance responses are important for the survival of shade-intolerant plants. Thus, Arabidopsis seedlings constantly monitor the R:FR ratio of the ambient light, which provides an unambiguous clue for the presence of close competitors. Low R:FR conditions are sensed by the phytochromes and trigger elongation responses and early flowering. The central repressor of light signalling, COP1, acts as a positive regulator of the elongation response of the hypocotyls to low R:FR signals. The four *SPA* genes code for repressors of photomorphogenesis that act together with COP1 in a complex. In this study, functions for the *SPA* genes in the elongation responses and the accelerated flowering were investigated.

III.1.1 *SPA* genes are essential for elongation responses in shade avoidance

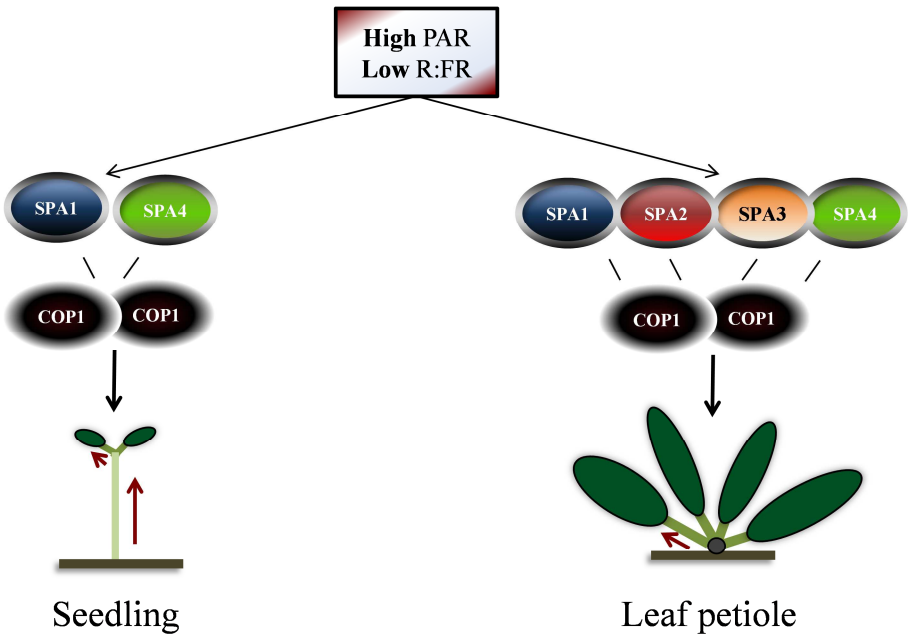

Figure III-1: *SPA* gene function in shade avoidance. A representation of the functions of the four *SPA* genes and *COP1* in the elongation responses to low R:FR. Red arrows indicate the elongation processes regulated.

COP1 is required for the elongation response of hypocotyls to low R:FR conditions and was shown to genetically interact with *BBX21* and *BBX22* (Crocco et al., 2010; McNellis et al., 1994). Our evidence shows that *SPA* genes are also essential for low R:FR - associated

elongation responses of the hypocotyl and of the petioles of cotyledons, as seedlings of the *spa1 spa3 spa4*, the *spa1 spa2 spa4* and the *spa-Q* mutants failed to respond to the low R:FR treatment (Fig. II-1; Dickopf, Master Thesis, 2011; Sahm, Examensarbeit, 2010). The hypocotyl elongation responses of all four *spa* single mutants and the double, triple and the *spa-Q* mutant revealed redundancy among the *SPA* genes, as no single *spa* mutant was defective in the low R:FR response. Also, differential functions of the *SPA* genes were observed. *SPA1* and *SPA4* are the main regulators of shade avoidance elongation responses, based on the phenotype of the *spa* triple mutants (Fig. III-1). Furthermore, the phenotypes suggest that *SPA4* is the major player of shade avoidance within the *SPA* gene family and that *SPA1* is contributing. Divergent functions have commonly been assigned to the *SPA* genes (Laubinger et al., 2004; Fittinghoff et al., 2006). Due to sequence similarity, *SPA* genes have been divided into the *SPA1/SPA2* and the *SPA3/SPA4* clade (Laubinger and Hoecker, 2003).

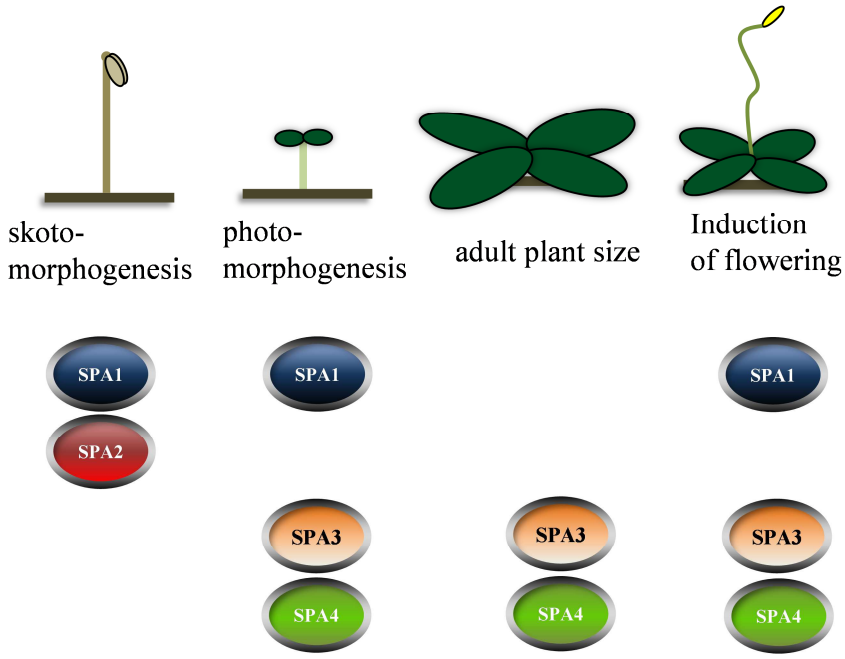

Figure III-2: Overlapping and distinct functions of SPA genes. *SPA* genes function throughout the lifecycle of Arabidopsis. The SPAs mainly involved in the control of the respective developmental stage are shown (Adapted from Fittinghoff, 2009).

While *SPA1* and *SPA2* are predominant in the repression of light signalling of dark-grown plants, *SPA1*, but not *SPA2* has a function in light-grown seedlings (Laubinger et al., 2004). Thus, low R:FR conditions do not simply resemble dark-like conditions, where *SPA1* and *SPA2* are the predominant factors, nor do they resemble FR light conditions, where *SPA3* has a function in addition to *SPA1* and *SPA4* (see fig. III-2; Laubinger et al., 2004). Low R:FR conditions rather represent a novel mode of differential *SPA* gene activity in seedlings, which resembles adult plant development and mostly the flowering time control, where *SPA1* is the

predominant gene and *SPA4* is contributing (Laubinger et al., 2006; Fackendahl, PhD Thesis, 2011). The differences in *SPA* gene function may result from differential regulation of the transcript levels, of the protein levels, different substrate specificity of the SPA proteins or additional different contribution to the stability and function of the COP1/SPA complex. The transcript levels of the *SPA* genes were determined and found to be unresponsive to the low R:FR conditions, which indicates that the difference in function in simulated shade is not solely due to differential regulation of the *SPA* genes or the transcript levels, but likely involves differences of the SPA protein activity (Fig. II-8). While *SPA1* transcript levels increased over time in both conditions, *SPA4* transcript levels did not increase, which could contribute to the *SPA1* function, but would not favour a function for *SPA4* in shade avoidance compared with *SPA2* and *SPA3*.

As SPA protein levels are controlled by light, differences in the accumulation to higher levels of the SPA proteins may contribute to their differential function (Balcerowicz et al., 2011). SPA1 protein levels were analysed, but no change in the protein levels in low R:FR conditions was observed, suggesting that SPA1 protein levels are not altered in response to low R:FR conditions (Fig. II-9). This indicates that other mechanisms are involved in the different activities of the *SPA* genes, given that SPA2 and SPA3 protein levels are not reduced by low R:FR conditions, which has not been addressed. In order to further unravel the contribution of the promoter activity and the protein function of SPA1 and SPA2, promoter-swap lines were analysed that express SPA1 or SPA2 from the *SPA1* or *SPA2* regulatory sequnces in the *spa-Q* background (Fig. II-7). While SPA1 rescued the *spa-Q* phenotype expressed from either promoter, SPA2 was able to function in the simulated shade conditions, but only when expressed from the *SPA1* regulatory sequences. Similar results have also been obtained for adult plant development in the light, but not for seedling phenotypes (Balcerowicz et al., 2011). It indicates that the inactivation of the SPA2 protein in the light in seedlings is partially reversed by the low R:FR conditions, but SPA2 activity is insufficient to cause SPA2 function in the *spa1 spa3 spa4* mutant background (Fig. II-2/II-7, Balcerowicz et al., 2011). SPA1 and SPA2 have previously been shown to be ubiquitously expressed in seedlings, but *SPA1* transcript accumulates to higher levels in light-grown seedlings than *SPA2* levels (Fig. II-8; Balcerowicz et al., 2011; Fittinghoff et al., 2006). As both promoters express ubiquitously and no regulation of *SPA1* and *SPA2* transcript levels was observed in low R:FR, it is likely that SPA2 protein function is threshold-dependent, as the SPA1 promoter expresses stronger than the SPA2 promoter (Fig. II-7, Fig. II-8, Balcerowicz et al., 2011). To support this view, the *SPA2* protein levels could be analysed in the *spa1 spa3 spa4*

mutant and compared with the protein levels in the WT and the *SPA1::SPA2* lines. SPA1 expressed from either promoter is functional, which suggests that the difference between SPA1 and SPA2 is partly conferred by the protein sequence, which might be due to protein stability, COP1/SPA complex activity or differential interaction with downstream targets. To address the possibility that different protein levels of SPA1 and SPA2 in light-grown seedlings may cause the difference between SPA1 and SPA2 function, lines that express comparable levels of SPA1 and SPA2, respectively, should be identified and analysed in the low R:FR conditions (Balcerowicz et al., 2011).

SPA1 and *SPA4* are the main regulators of the low R:FR elongation responses of seedlings and both likely act in a dose-dependent manner in the elongation response to low R:FR, as lines that were previously shown to overexpress SPA4, led to over-complementation of the mutant phenotype, which was also observed for SPA1 in this study and previously (Fig. II-14, Fig. II-15; Dickopf, Master Thesis, 2011; Fackendahl, PhD Thesis, 2011). The coiled-coil domain of SPA4 was essential for *SPA4* function in the hypocotyl elongation in response to low R:FR conditions (Fig. II-14). This suggests that the interaction of SPA4 with other SPAs or COP1 or both is required for *SPA4* function in shade avoidance, which adds on the compelling evidence for common COP1 and SPA functions that form the COP1/SPA complex dependent on the cc-domain interface (Zhu et al., 2008). Given that overexpression of SPA proteins is capable of over-complementing the elongation response of the hypocotyl, the SPA protein levels are a limiting factor for the low R:FR response of Arabidopsis (Figures II-14 and II-15). This further supports the notion that SPA genes are important positive regulators of the elongation response. The overexpression of SPA1 and SPA4 may cause over-complementation, because the amount of SPA1/4 containing complexes is expected to be increased compared with other residual COP1/SPA complexes, which may increase the activity of the complexes in the elongation responses. To further support the function of *SPA1* and *SPA4* and show a lack of function of *SPA3* and *SPA4*, I attempted to isolate a *spa1 spa4* and a *spa2 spa3* double mutant from crossings, but the detection of correct mutants failed (data not shown). The newly available null mutants, *spa1-100*, *spa2-2* and *spa4-3*, could be used for a new round of shade avoidance experiments to rule out effects from truncated proteins still expressed as was shown for SPA2 in the *spa2-1* mutant (Zhu et al., 2008).

SPA genes and *COP1* are both required for the elongation responses of adult leaf petioles (Fig. II-4 see also Fig. III-1). While the *spa-Q* lacked a response of the petiole to the low R:FR treatment, all *spa* triple mutants retained the elongation response. This is in agreement with the observation that *SPA2* has no function in light-grown seedlings, but a minor function

in adult plants that can even be strengthened by expressing SPA2 under the control of the *SPA1* regulatory sequences (Laubinger et al., 2004; Fittinghoff et al., 2006; Balcerowicz et al., 2010). All *SPA* genes contributed to the elongation of the petioles of true leaves, which contrasts the seedling phenotypes of the *spa* triple mutants that showed a differential contribution of the *SPA* genes to the low R:FR response. This could be explained with the action of different elongation promoting factors downstream to the COP1/SPA complex in true leaf petioles compared with the hypocotyl of seedlings or differences in the abundance and activity of COP1/SPA complexes in seedlings and adult plants. Though some genes, such as certain *XHT* genes, are only up-regulated in the true leaf blade and/or petiole and not in the seedling in response to low R:FR, the important shade marker genes, such as *HFR1* and *ATHB2* are also up-regulated in the adult leaves, which suggests largely similar mechanisms of the two elongation responses (Devlin et al., 2003; Kozuka et al., 2010). Thus, the difference in function of *SPA2* and *SPA3* in seedlings and adult leaves may be due to differences of the activity of the COP1/SPA complexes. However, it was shown that SPA1 has different functions in different tissues and the SPA/COP1 complex may serve a different function in the two processes (Ranjan et al., 2011).

As thoroughly discussed by Fackendahl (2011), the *spa* triple mutants and the *spa-Q* mutant used in this study only contain one true null-allele, *spa3-1*, while the other *spa* mutant alleles are not considered to be null alleles. Thus, the *spa* mutants may still express truncated versions of the SPA proteins, which was shown for *spa2-1*, which still expresses a truncated SPA2 protein (Laubinger and Hoecker, 2003: Zhu et al., 2008).The difference in seedling and adult elongation phenotype may not reflect a difference in *SPA2* function in these processes, but could reflect a different influence of the N-termini of SPA1 or SPA4. There is evidence that the N-terminus of SPA1 is involved in the control of flowering, but not at the seedling stage (Fittinghoff, PhD Thesis, 2009). However, more recent observations indicate that the N-terminus of SPA1 might also be contributing in certain *spa* mutant backgrounds, also in the seedling (Dieterle, S., unpubished data; Fackendahl, PhD Thesis, 2011).

COP1 and other components of the shade avoidance elongation response pathway have been discovered in a genome wide association study (GWAS) with 180 Arabidopsis genotypes, but *SPA* genes were absent from the list of genes associated with shade elongation response of the hypocotl (Filiault and Maloof, 2012). This may reflect an unexpected invariability of *SPA* genes in the accessions included or might be due to the redundancy in the *SPA* gene family, as single mutants in the Col-0 background do not exhibit defects in the shade avoidance responses (Fig. II-2 A,B). However, the *spa1-2* single mutant displayed a reduced elongation

response to the low R:FR conditions, which indicates that single *SPA* genes may be limiting for the shade avoidance response in certain Arabidopsis ecotypes or that certain mutations in *SPA* genes can elicit aberrant shade-related phenotypes compared to Col-0 (Fig. II-13).

III.1.2 *SPA* gene and *COP1* are not involved in the acceleration of flowering in response to low R:FR conditions

Prolonged shade conditions lead to an acceleration of the transition from vegetative to reproductive growth, which is anticipated by an increase of *FT* transcript level well before the development of flowers (Cerdán and Chory, 2003; Wollenberg et al., 2008). The COP1/SPA complex acts in the photoperiodic flowering pathway, negatively influencing *FT* expression by the repression of CO protein levels in SD conditions (Laubinger et al., 2006; Jang et al., 2008). Accordingly, the acceleration of flowering in simulated shade in the *spa* triple mutants, the *spa-Q* and *cop1-4* mutant was analysed (Fig. II-5). All mutants flowered at the same time and with the same number of leaves compared to the WT in low R:FR conditions. The *FT* transcript levels were uniformly up-regulated in response to prolonged low R:FR conditions compared with the Wc conditions in the WT, the *spa1 spa3 spa4* mutant and the *cop1-4* mutant, which is consistent with the similar flowering time phenotypes in low R:FR (Fig. II-6). The *CO* transcript levels were reported to increase shortly after the onset of low R:FR conditions (Kim et al., 2008) and showed a minor regulation in response to the shade treatment in the *spa1 sp3 spa4* mutant and *cop1-4* mutant. No significant regulation of CO was observed in the WT. As the expression of *CO* is regulated by the circadian clock, the WT and the mutants may differ in the timing of the *CO* regulation or the regulation may generally differ in the backgrounds (Kim et al., 2008). Nevertheless, these differences in *CO* transcript levels are not dramatic and do not result in different *FT* transcript levels or altered flowering time phenotypes. In order to investigate, whether CO regulation is generally different in WT and the *spa* triple mutant and *cop1-4*, additional different time-points could be analysed as *CO* transcript levels are regulated by the clock with the highest differences between 12 hour time-points (Imaizumi et al., 2003). *FLC* transcript levels were highly up-regulated in the *cop1-4* background with correlates with the lower overall *FT* levels in the *cop1-4* mutant. FLC levels were reported to be elevated up to 5-fold in the *spa1 spa3 spa4* and the *spa2 spa3 spa4* compared with the WT dependent on the time of day in SD (Laubinger et al., 2006). In order to unravel, if *FLC* transcript levels are up-regulated in a *cop1* dependent manner, additional *cop1* mutants also from other ecotypes could be analysed in parallel. Furthermore, the high *FLC* levels may be connected with the constitutive photomorphogenic phenotype, thus the

constitutive photomorphogenic *spa1 spa2 spa4* mutant should be tested accordingly. Thus, it is concluded that neither the *SPA* genes, nor *COP1* are involved in the acceleration of flowering time in the low R:FR environment. It had already been proposed that phyB may control CO protein levels independently of COP1 and could act on flowering time in a COP1 independent fashion (Jang et al., 2008). Furthermore, SPA1 represses flowering in SD when expressed in the phloem, while phyB acts in the mesophyll to inhibit flowering, which likely places the COP1/SPA complex downstream of cry function in flowering time control and is likely to be independent of phyB input (Endo et al., 2005; Ranjan et al., 2011). The presence of *CO* was shown to be important for the acceleration of flowering, as *co* mutants are impaired in the early flowering in response to low R:FR (Wollenberg et al., 2008). To further support the notion that *COP1* and the *SPA* genes are not involved in the acceleration of flowering, the genetic interaction of *CO* and *COP1* and *SPA1* in the flowering time cold be analysed with the *cop1-4 co* and the *spa1 co* mutants in our simulated shade conditions (Laubinger et al., 2006; Jang et al., 2008).

Taken together with the *SPA* and *COP1* function in the elongation responses to low R:FR, these results also support the hypothesis that two distinct molecular pathways of different evolutionary origin operate in the shade avoidance syndrome, as the elongation responses and the acceleration of flowering time are unrelated downstream of phyB (Botto and Smith, 2002).

III.1.3 *SPA* genes interact with a negative regulator of low R:FR signalling

HFR1 is a negative regulator of shade avoidance that inhibits PIF proteins to prevent overstimulation of the shade avoidance responses (Hornitschek et al., 2009; Sessa et al., 2005). COP1 and SPA1 physically interact with HFR1 and regulate HFR1 levels during photomorphogenesis (Duek et al., 2004; Yang et al., 2005a/b).Therefore, it was tested, if *SPA* genes may also interact with *HFR1* genetically in the elongation response to low R:FR of the seedlings. A genetic interaction between *SPA* genes and *HFR1* in the hypocotyl elongation response to low R:FR conditions could be observed, as introduction of the *hfr1* mutation into the the *spa1 spa3 spa4* mutant restored the elongation response to low R:FR (Fig. II-10; Sahm, Examensarbeit, 2010).

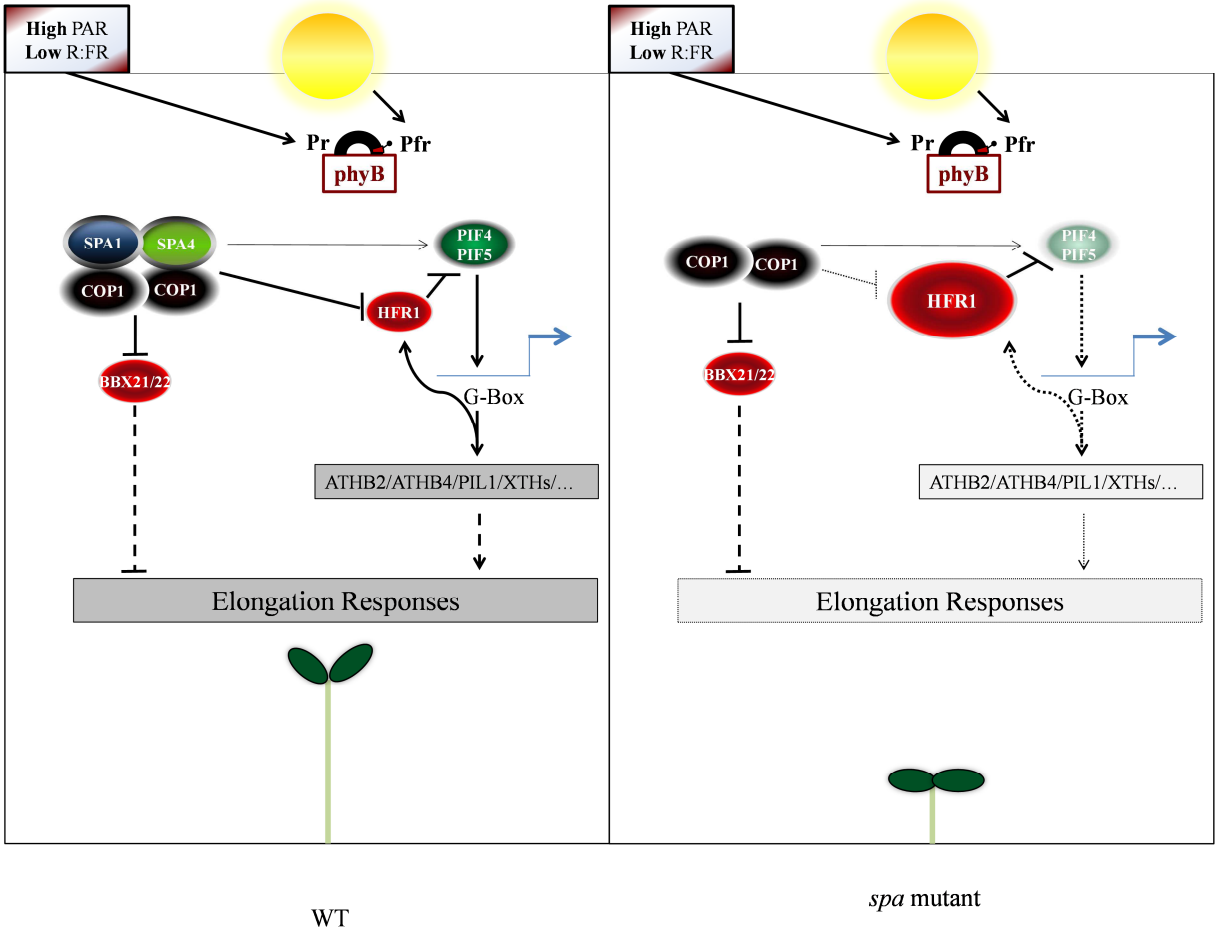

Figure III-3: Model of SPA function via HFR1 in low R:FR. In the WT background, SPAs acts on HFR1 levels to prevent over-accumulation, which positively acts on the PIF-pathway. In spa mutant backgrounds, HFR1 may overaccumulate and the PIF pathway blocked. Also, *COP1* acts on BBX proteins that inhibit elongation.

It is hypothesised that HFR1 over-accumulation causes the lack of elongation response of multiple *spa* mutants and the *cop1-4* mutant (see Fig. III-3). The differential function of *SPA1* and *SPA4* in shade avoidance together with the genetic interaction of *HFR1* fostered the speculation that differential interaction of SPA proteins with HFR1 may account for the differences in *SPA* function. The results accumulated in two recent studies do not allow a clear conclusion, whether or not SPA proteins differentially interact with HFR1, because the data are contradictory (Dickopf, Master Thesis, 2011; Meller, Master Thesis, 2011).

It has been stated that mutants deficient in single components of the shade avoidance signalling network exhibit mostly mild effects due to the complexity of the signalling network (Galstyan et al., 2011). The complete lack of elongation responses in the seedlings of two *spa* triple mutants and the *spa-Q* may seem unlikely to solely result from absence of the regulation of HFR1 protein levels in these mutants, as HFR1 negatively acts on PIF4 and PIF5 and the *pif4 pif5* double mutant still exhibits a pronounced elongation response (Lorrain et al., 2008). This would indicate that the *SPA* genes act on additional target genes or that HFR1 would act

on additional targets others than PIF4 and PIF5 specifically in shade conditions. The N-terminus of HFR1 is important for HFR1 stability and its N-terminus and bHLH domain are important for the interaction with COP1, while interaction with SPA1 depends on the presence of several domains (Yang et al., 2005a/b; Duek et al., 2004). Overexpression of the bHLH domain alone or the bHLH domain together with the C-terminal part of HFR1 causes severe reduction of the hypocotyl elongation in response to low R:FR treatment compared with the WT and leads to partial photomorphogenesis in darkness (Galstyan et al., 2011; Yang et al., 2003). Thus, HFR1 fragments, which lack the regulatory N-terminal domain for degradation by the COP1/SPA complex, cause a strong reduction of the elongation response of the WT, while overexpression of full length HFR1 causes only mild phenotypes in the response to low R:FR conditions. These findings are in agreement with the notion that over-accumulation of HFR1 in the *cop1* and *spa* triple and quadruple mutants may cause the severe seedling phenotypes observed in low R:FR conditions compared with the WT.

HFR1 and *COP1* have been proposed to affect the expression of shade marker genes previously. The transcript levels of *PIL1* have been reported to be elevated in the *hfr1* mutant and reversely reduced after 3h in both, *pif4 pif5* double and *pif4 pif5 hfr1* triple mutants specifically in response to low R:FR (Hornitschek et al., 2009). In the same study, *XTR7* has also been shown to be antagonistically regulated by *HFR1* and *PIF4* / *PIF5*. PIF5 directly binds to the *XTR7* and *PIL1* promoters and the binding is inhibited by HFR1 (Hornitschek et al., 2009). Furthermore, the transcript levels of *HFR1* were lower in *pif4 pif5* mutants (Lorrain et al. 2008). Overexpression of truncated HFR1 inhibits the accumulation of *PIL1* transcript levels after 1h of shade treatment in 7-day-old plants and a milder effect was observed in HFR1-HA overexpression lines (Galstyan, 2011). Also, *COP1* has been reported to negatively act on the increase of *ATHB2* and *PIL1* transcript levels in response to low R:FR, which is in agreement with a *COP1* function upstream of *HFR1* (Roig-Villanova et al., 2006). On the contrary, *PIL1* transcript levels were found to increase similarly in the WT and *cop1* mutant in short term shade and also *ATHB2* and *HFR1* transcript levels were only slightly affected by *COP1* in a different study (Crocco et al., 2010). In this study, the up-regulation of the transcript levels of *PIL1*, *ATHB2* (three hours of low R:FR treatment) and *HFR1* (24 hours and 48 hours) were similar in the *spa* multiple mutant seedlings in response to low R:FR conditions compared to the WT. Also, *HFR1* levels were equally up-regulated in the *cop1-4* background compared to the WT. This contradicts the proposed function for *COP1* in the general regulation of early shade marker genes (Roig-Villanova et al., 2006).

The *PIL1* transcript levels were strongly increased in response to the low R:FR treatment in the WT, the *spa1 spa3 spa4* mutant and the *cop1-4* mutant. No difference in the transcript levels of *PIL1* was observed between the genotypes in the time-course experiment that covered 48 hours of low R:FR treatment (Fig II-3 C). On the one hand, this would contradict the notion that HFR1 may over-accumulate in two of the multiple *spa* mutants and the *cop1* mutant, as *HFR1* has repeatedly been reported to act on the transcript levels of *PIL1* and *ATHB2* in a low R:FR-specific way (Hornitschek et al., 2009; Sessa et al., 2005). On the other hand, the transcript levels of *XTR7* were found to be differentially regulated between the WT and the *spa* triple mutants in response to low R:FR conditions. It was shown previously that overexpression of a ΔN-HFR1 construct suppresses *XTR7* expression, which leads to shorter hypocotyls in darkness (Yang et al., 2003). Furthermore, in this study, *ATHB2* transcript levels in low R:FR and Wc were analysed in the *hfr1* mutant and the *spa1 spa3 spa4 hfr1* quadruple mutant. In response to low R:FR treatment, the *ATHB2* levels increased strongly in all backgrounds tested and no over-accumulation of *ATHB2* transcript was detected in the *hfr1* mutant background compared to the WT (Fig- II-10 C). This indicates that the reported *HFR1* function on the expression of *ATHB2* is not detectable in our shade setup. This supports the notion that *SPA* regulation of *HFR1* may not be detected on the level of transcript level of shade marker genes, apart from the *XTR7* levels.

Taken together, *SPA* genes acted differentially on the expression of shade marker genes. The difference in regulation of *PIL1* in the different studies could be explained by additional, PIF-independent, mechanisms that may override the *HFR1* function in specific low R:FR conditions, as it was reported that the binding of PIF factors to the G-Boxes of the *PIL1* promoter are not the only factors that influence *PIL1* expression in response to low R:FR conditions (Li et al., 2012). Furthermore, as *ATHB2*, *PIL1* and *HFR1* transcript levels were strongly up-regulated in the *spa* multiple mutants, a functional significance for the increase in the transcript levels of these genes in the elongation responses to low R:FR is to be questioned. XTR7/XTH15 is up-regulated swiftly in short-term shade and remains up-regulated in long-term shade, which is rare among the *XHT* genes. *xtr7* mutants display no induction of growth rate of the petiole in low R:FR conditions (Sasidharan et al., 2010). As the shade-induction of *XTR7* is directly associated with the increased elongation of seedlings, the transcript levels in low R:FR conditions can be correlated with the observed hypocotyl and cotyledons phenotypes of the *spa* mutants, with the exception of the *spa2 spa3 spa4* mutant that exhibits an elongation response to low R:FR conditions, but lower *XTR7* levels in low R:FR compared to the WT and the *spa1 spa2 spa3* mutant (Fig. II-3 A).

Further evidence to support the hypothesis that over-accumulation of HFR1 may cause the observed elongation phenotypes of the *spa* mutants could derive from the determination of HFR1 protein levels in *spa* triple and *spa-Q* mutant backgrounds. No HFR1 antibody is available to date, so lines that express tagged versions of HFR1 could be crossed into the *spa* and *cop1* mutant backgrounds to compare protein levels in Wc and low R:FR conditions between the mutants and the WT (HFR1-HA; Jang et al., 2005).

COP1 was shown to act on two B-BOX transcription regulators of shade avoidance, *BBX21* and *BBX22* (Crocco et al., 2010). Also, *BBX21/STH2* was shown to act downstream to *SPA* genes in the control of adult plant growth (Fackendahl, PhD Thesis, 2011). Yeast-two hybrid protein-protein interaction studies revealed a putative interaction of SPA4 with BBX21 (Falke, Master-Thesis). Taken together, it is likely that SPA proteins negatively regulate the function of BBX21 and presumably BBX22 in regulation of shade avoidance responses, which could be further investigated by the analysis of crosses with *spa* triple mutants and the *bbx21/bbx22* single mutants and the determination of BBX protein levels in the mutants. Taken together, the data suggest that the COP1/SPA complex acts on negative regulators of the elongation responses to low R:FR conditions, such as HFR1 and BBX21/22 to control the negative feed-back on elongation (Fig. III-3).

HY5 is targeted for degradation by the COP1/SPA complex in darkness, which inhibits HY5 function that in turn promotes photomorphogenesis when the inhibition by COP1 is released in the light (Osterlund et al., 2000; Saijo et al., 2003). HY5 was recently described to be up-regulated in response to a sunfleck treatment, which is characterised by a rapid and transient reversion of low R:FR conditions to high R:FR that inhibit the low R:FR triggered hypocotyl elongation (Sellaro et al., 2011). HY5 counteracts the shade avoidance machinery in response to sunfleck conditions. Thus, over-accumulation of HY5 in the *spa* mutant backgrounds could contribute to the lack of elongation response observed (Fig. II-10 A,B). In our simulated shade conditions, no genetic interaction of *SPA* genes and *HY5* or *COP1* and *HY5* could be observed, as *hy5* mutations in the *spa1 spa3 spa4* background and the *cop1-4* background rescued the Wc hypocotyl phenotype of the mutants, but did not restore the elongation response to simulated shade. The function of *HY5* likely depends on the fact that in natural light-dark-cycles, *HY5* transcript levels are highly up-regulation in sunfleck conditions. Furthermore, in natural conditions, HY5 is up-regulated at dawn even in shade conditions, which is different from our continuous light conditions (Sellaro et al., 2011). Hence, it is conceivable that COP1/SPA may be important to control HY5 protein levels in shade and sunfleck conditions in light/dark cycles to prevent over-accumulation of HY5, but not in our

simulated shade conditions. To test this hypothesis, it should be attempted to elicit sunfleck responses in WT seedlings with modifications of the low R:FR setup and reproduce the results obtained for *HY5* in sunfleck. The sunfleck response could then be analysed in *spa* mutants and the *cop1-4* mutant and in the *cop1 hy5* double mutant and the *spa1 spa3 spa4 hy5* mutants to check for a genetic interaction of *COP1* and *HY5* and *SPA* genes and *HY5* in the sunfleck response.

III.1.4 Genetic interaction of photoreceptors with *SPA* genes and *COP1* in shade avoidance

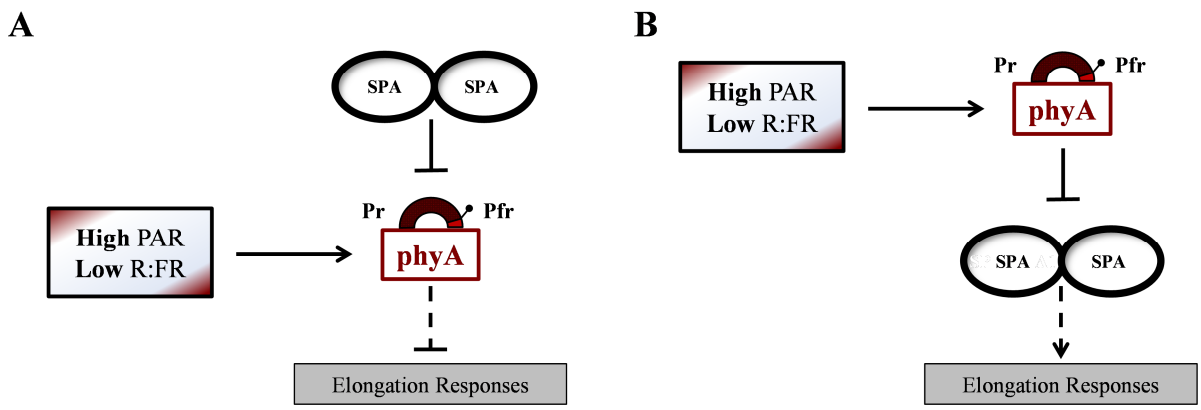

Figure III-4: Model of the interaction of SPAs and phyA in low R:FR. phyA is activated in low R:FR and acts negatively on the elongation responses. The SPAs may act upstream of phyA to negatively regulated phyA signalling **(A)** or could act downstream of phyA **(B)**.

The COP1/SPA complex is a mediator of light signals that are perceived by the photoreceptors and downstream transcription factors that are repressed in the absence of light stimuli. It was shown to interact with phyA, phyB and the cryptochromes and functions in B, R and FR light signalling (Jang et al., 2010; Lian et al., 2011; Liu et al., 2011; Seo et al., 2004; Wang et al., 2001; Yang et al., 2001). Between the Wc conditions applied in this study and our low R:FR conditions, which are enriched FR conditions on top of the Wc setting, the two phytochromes phyB and phyA are antagonistically regulated. phyB is largely transferred to the inactive Pr from, while phyA is stabilized and stimulated by the FR fluence-enriched environment. This is reflected by the phenotype of the phyA mutant that exhibited exaggerated hypocotyl lengths in the shade conditions compared with the WT and the phyB mutant, which displays shorter hypocotyls in the low R:FR compared with the Wc conditions, but elongated hypocotyls in Wc (Fig. II-11-13). As *cop1-6 phyB* double mutants resemble *cop1-6* mutants, the *phyB*-9 phenotype is completely repressed by *cop1*, which places *COP1*

downstream of phyB. This indicates that the inactivation of phyB is mainly acting on elongation responses via COP1 (Fig. II-12).

While *spa1 spa3 spa4* mutants failed to respond to low R:FR treatment *phyA spa1 spa3 spa4* mutants exhibited elongated hypocotyls in response to low R:FR, which means that introduction of the *phyA* mutation into the *spa* triple background restored the elongation response of the mutant (Fig. II-11). This suggests that the *spa* mutant is hypersensitive to phyA signalling, which is absent from the phyA mutant. This could be due to phyA over-accumulation in the mutant, as COP1/SPA is shown to act negatively on phyA signalling (Seo et al., 2004). Alternatively, phyA may more efficiently inactivate COP1/SPA2 complexes than other COP1/SPA complexes, which would lead to a loss of activity of the COP1/SPA complex. These two possibilities are presented in figure III-4. In order to discriminate between them, it would be helpful to examine the *cop1 phyA* or *spa1 spa2 spa4 phyA* shade phenotypes, as these mutants are constitutively photomorphogenic, so no input from the phytochromes is expected and the mutants should be independent of phyA input, while *spa1 spa3 spa4* is still responsive to phyA signalling due to SPA2, which functions in darkness to repress photomorphogenesis (Laubinger et al., 2004).

Previously it was shown that *SPA1* is fully epistatic over *PHYB* in the development of the leaf blade (Ranjan et al., 2011). Analysing the interaction of *SPA1* with *PHYA* and *PHYB*, it could be seen that the *spa1-2* mutation only had mild effects in the backgrounds of the *phyA* and *phyB* mutants (Fig. II-13). As *spa1-2* is not fully epistatic over *phyB* in the elongation response of the hypocotyl to low R:FR conditions, the phyB-dependent leaf expansion and the phyB-dependent hypocotyl elongation should be considered as two distinct pathways. *SPA1* has a more prominent function in the leaf expansion than in the elongation response of the hypocotyl downstream of phyB (Fig. II-13; Ranjan et al., 2011).

III.1.5 *SPA1* expression from the *ML1* and *CER6* promoters triggers an elongation response to low R:FR in the *spa1 spa2 spa4* mutant background

Tissue-specific functions of SPA1 were discovered previously (Ranjan et al., 2011). Expression of SPA1 from the *SUC2* promoter represses the constitutive photomorphogenic phenotype of dark-grown *spa* mutants and causes an increase of the hypocotyl length in darkness and the light. Furthermore, SPA1 expression in the phloem restores proper flowering time and controls leaf size. Expression in the epidermis with the *ML1* promoter in darkness and the *ML1* and *CER6* promoter in the light has only mild effects compared to the expression from the *SUC2* promoter in the *spa1 spa2 spa3* mutant background (Ranjan et al., 2011).

Expression from the *ML1* and *CER6* promoters did not complement the hypocotyl phenotype in Wc, but restored the elongation response of the *spa* triple mutant (Fig. II-15 A,B). Provided that low R:FR conditions do not alter the expression pattern of *ML1* or *CER6* and that SPA1 functions in the tissue it is expressed in and does not move, it can be concluded that expression of SPA1 in the epidermis was sufficient to rescue the elongation response to low R:FR in the *spa1 spa3 spa4* mutant. Both genes (*ML1* and *CER6*) are stably expressed in different light conditions, which makes it likely that they uphold their expression pattern in response to low R:FR, but it will be necessary to investigate the GUS staining in the Wc and low R:FR conditions on a cellular level to rule out the possibility that ectopic expression is responsible for the rescue of the shade phenotype of the hypocotyl. The data reveal that the site of SPA1 function in shade avoidance related elongation responses of the hypocotyl differs from darkness- and light-regulated elongation responses of the hypocotyl. This suggests a function of SPA1 that is independent from its function in photomorphogenesis in the vascular tissue. Thus, an additional and likely distinct tissue-specific function was discovered by the results obtained in this study. The epidermis is involved in the regulation of plant growth and drives elongation responses (Savaldi-Goldstein et al., 2007). This involves BR signalling. Hence, one hypothesis is that SPA1 in the epidermis could act on the BR signalling pathway, which is implicated in the elongation responses of the hypocotyl. BR biosynthesis mutants fail to elongate the hypocotyl in response to low R:FR (Luccioni et al., 2002). Furthermore the BR pathway is under negative control of the photoreceptors, which could place the COP1/SPA complex downstream of the photoreceptors and upstream of BR biosynthesis or signalling (Vandenbussche et al., 2005). Also the expression of *XTR* genes is under the control of BR and auxin signalling and *XTR7* transcript levels are reduced in *spa* mutants compared to WT in low R:FR conditions. Alternatively, redistribution of auxin to the epidermis and auxin signalling in the epidermis are important for the elongation response of the hypocotyl and SPA1 may be important for the auxin response in the epidermis (Keuskamp et al., 2010).

III.2 Phenotypes of *spa* mutants correlate with auxin signalling

Auxin is involved in cell elongation and proliferation and determines final organ size and shape. The dwarfed phenotype of *cop1* and multiple *spa* mutants from the seedling stage to adult plants may reflect an aberrant regulation of the auxin pathway in the *spa* mutants.

Furthermore, non-cell-autonomous functions have been assigned to SPA1, which makes it likely that *SPA* genes act on hormonal pathways, such as auxin biosynthesis, transport or signalling.

III.2.1 Auxin response in *spa1 spa3 spa4* and *spa1 spa2 spa4* seedlings

Auxin response in the hypocotyl and the cotyledons is under the control of the photoreceptors that act on auxin biosynthesis and transport (Hoecker et al., 2004; Salisbury et al., 2007; Tao et al., 2008). Auxin-transport is not necessary for elongation of seedlings in darkness and the PAT is under tight control in the light, which coordinates shoot and root development (Salisbury et al., 2007). Apart from that, light is thought to act on the responsiveness of the tissues to auxin (Cluis et al., 2004; Nozue et al., 2011; Sibout et al., 2006).

Taken together, the auxin response differs in dark-grown and light-grown seedlings, which contributes to the skotomorphogenic and photomorphogenic phenotypes.

It was tested, whether two *spa* triple mutants showed altered auxin response in seedlings in darkness and low FR light (Fig. II-16). While the WT showed high auxin response in the cotyledons in darkness, auxin response was mostly observed to be constraint to the root tip in the s*pa1 spa3 spa4* and *spa1 spa2 spa4* mutants. This indicates that the auxin response in the mutants is already lowered in darkness in both mutants, though the *spa1 spa3 spa4* mutant retains skotomorphogenesis comparable to the WT. This indicates that *SPA* genes act positively on auxin response in darkness. In the light, the photoreceptors may inhibit this *SPA* function. To further study the responsiveness of the *spa* mutants to auxin, dose-response curves with NAA could be carried out comparing the *DR5::GUS* response to exogenous auxin in WT and *spa* mutant backgrounds in darkness and light. Also, the hypocotyl elongation responses in response to the auxin treatment should be correlated with the results of the NAA dose-response curves.

III.2.2 *SPA* genes are involved in the increase of auxin response in response to low R:FR

Auxin is essential for the low R:FR triggered elongation responses. Auxin levels are elevated in low R:FR conditions, which likely results from the up-regulation of *YUC* genes that act downstream of *TAA1* (Tao et al., 2008; Won et al., 2011). Also, the auxin response is elevated in the cotyledons of seedlings in response to simulated shade (Tao et al., 2008). A recent publication places PIF7 directly upstream of *YUC* gene expression specifically in low R:FR conditions (Liu et al., 2012). The auxin response was analysed in the *spa1 spa3 spa4* and *spa1 spa2 spa4* mutant backgrounds that both failed to respond to low R:FR treatment at the

seedling stage. At 27°C, overall auxin levels are elevated, which facilitates the visualisation of the increase of the auxin response in the WT background (Franklin et al., 2011). Nevertheless, the elongation response on top of the increased hypocotyl length is comparable to 21°C (Fig. S2). The increased auxin response that could be observed in the *DR5::GUS* line, was absent from the *spa* triple mutant *DR5::GUS* lines, which indicates that the auxin response is not increased in these background and which correlates with the aberrant elongation phenotypes in low R:FR (Fig. II-17).

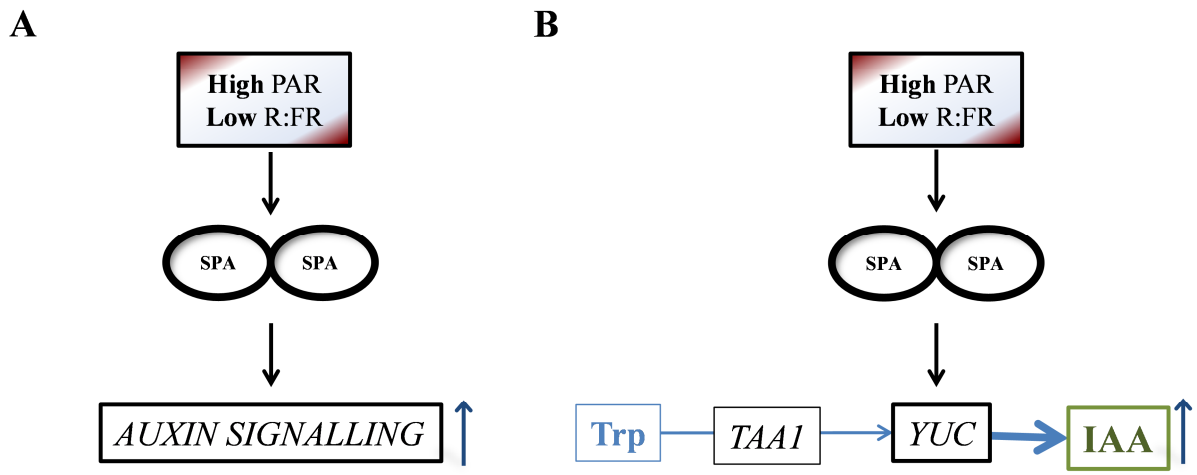

Figure III-5: Model of SPA function in auxin signalling in response to low R:FR. A) SPAs are important for the elevated auxin signalling in low R:FR conditions **B)** *SPA* genes act on the expression of auxin biosynthesis genes (shown for *YUC8*) to elevate auxin levels.

The data could be explained with a lack of an increase of the auxin biosynthesis in the *spa* mutant backgrounds, as it is established that in response to low R:FR, auxin levels are elevated, which leads to the increase of *DR5* activity (Tao et al., 2008). Indeed, this study points towards a close link between *SPA* gene function and the control of auxin biosynthesis. The transcript levels of *YUC8*, an auxin biosynthesis gene reported to be up-regulated by low R:FR treatment in seedlings and in the leaf petioles, showed similar levels in Wc and Wc+FRc in the *spa1 spa3 spa4* triple mutant background compared to the WT in response to low R:FR (Fig. II-18 ; Kozuka et al., 2010; Tao et al., 2008). The same trend could be observed for *YUC9*, but the induction in response to the low R:FR treatment is not significant in the WT. As has been reported, the transcript level of *TAA1* was unresponsive to the shade treatment in the WT and also in the *spa* triple mutant and *cop1-4* (Fig. II-18 B). This leads to the conclusion that at least one auxin biosynthesis gene is differentially regulated in one of the *spa* triple mutant background that displays no elongation response of the hypocotyl. On the

other hand, it had been reported that the *yuc8 yuc9* double mutant displays a normal response to simulated shade avoidance (Tao et al., 2008). However, a second study has provided evidence that a *yuc3 yuc5 yuc7 yuc8 yuc9* multiple mutant exhibits a weaker elongation response to low R:FR treatment and that auxin biosynthesis genes are direct targets of PIF7, which has been assigned a novel function in shade avoidance (Li et al., 2012). Furthermore, a *yuc1 yuc4* double mutant exhibited a reduced elongation response of the hypocotyl to low R:FR conditions compared to the WT (Won et al., 2011). The transcript levels of *YUC8* and *YUC9* and additionally of *YUC1*, *YUC3*, *YUC4*, *YUC5* and *YUC7* should be tested at the seedling stage in white light and shade in the WT and *spa* triple and quadruple mutants to further investigate the regulation of auxin biosynthesis genes reported to be involved in shade avoidance. A function for *YUC* genes in the response to low R:FR conditions is further supported by evidence from a genome wide association study (GWAS), that discovered the two genes, *YUC8* and *YUC9* as important genes for the elongation response to low R:FR conditions traits (Filiault and Maloof, 2012).

Apart from their putative action on auxin biosynthesis, *SPA* genes may also be involved in the control of auxin transport and the manipulation of auxin signalling by differentially regulating auxin-response genes. In contrast to the *DR5::GUS* results obtained in this study, the transcript levels of *IAA19* were up-regulated in response to three hours of low R:FR treatment in the *spa1 spa3 spa4* mutant. This may indicate that *IAA19* expression is not solely dependent on auxin (see chapter II.3) in these conditions or that auxin signalling increases in the *spa1 spa2 spa4* background, but does not reach the threshold necessary for the *DR5::GUS* detection. It can also not be ruled out that the elevation of auxin levels at the higher temperature may be absent in the *spa1 spa2 spa4* and the *spa1 spa3 spa4* mutant. Nevertheless, also results obtained at 21°C degrees pointed towards an elevation of *DR5::GUS* activity specifically in the WT in response to low R:FR conditions.

III.2.3 Auxin signalling in young leaves of *spa1 spa3 spa4* and *spa1 spa2 spa4* mutant plants

It was shown that cell size and cell number of dwarfed *spa1 spa3 spa4* leaves is diminished, which could be connected to altered auxin response in the developing tissues (Fackendahl, PhD Thesis, 2011).

The GUS activity was measured from the youngest leaves of two week-old *DR5::GUS, spa1 spa3 spa4 DR5::GUS* and *spa1 spa2 spa4 DR5::GUS* plants (Fig. II-19.). The GUS activity was significantly reduced in the *spa1 spa3 spa4 DR5::GUS* leaves, but comparable in

DR5::GUS and *spa1 spa2 spa4 DR5::GUS* plants, which correlates with the leaf sizes of the three genotypes. This indicates that auxin response in *the spa1 spa3 spa4* mutant background is reduced, which could cause the lower elongation and proliferation of cells in the leaves, which results in the dwarfed phenotype. The difference in auxin response between young leaves of the *spa1 spa3 spa4* mutant and the bigger *spa1 spa2 spa4* mutant points towards a function of SPA3 in the regulation of the auxin response that cannot be compensated for by SPA2. Total auxin levels could be determined in the *spa* triple mutants to correlate auxin levels with the observed phenotypes. Furthermore, the transcript levels of auxin biosynthesis genes could be determined in the mutants. Also, the localisation of PIN proteins and the expression of auxin signalling components could be analysed in the mutant backgrounds to gain further insight into the causes of the altered auxin response observed in the *spa1 spa3 spa4* mutant background. However, the reduced auxin response in the *spa* triple mutant could be a secondary effect in the *spa* mutants e.g. reduced leaf size itself, as the young leaves and the shoot apex are the main sources of auxin. Thus the auxin supply might be limited as a consequence of the phenotype and not vice versa.

III.2.4 COP1/SPA inhibits the root elongation of dark-grown seedlings in an NPA dependent way

Dark-grown *spa1 spa2 spa4* mutants and the *cop1-4* mutants largely resemble light-grown WT seedlings. This holds true for the elongation of roots in darkness as well, as roots keep extending in dark conditions compared to the WT (Fig. II-21/22; Dyachok et al., 2011; McNellis et al., 1994). It was hypothesised that auxin may contribute to the extension of the roots, so the seedlings were treated with auxin transport inhibitor (NPA). The application of NPA was able to reverse this extension, while the WT root was unaffected by the NPA treatment (Fig. II-21 A-C). Furthermore, the root growth in the light can be inhibited by NPA treatment to the same extend in the WT, the *spa1 spa2 spa4* mutant and the *cop1-4* mutant, which indicates that the effect of *COP1* and *SPA* on root extension prevented by NPA was restricted to darkness.

As light controls the shoot to root transport of auxin downstream of phyA and phyB by acting on PIN3 auxin efflux carrier function, auxin transport into the root in the *spa* an *cop1* mutants may contribute to the root elongation in darkness (Salisbury et al., 2007). Through PIN proteins, auxin gradients are also established within the root, which regulate cell elongation and proliferation. PIN2 is stored in root cells in darkness and moves to the membrane triggered by light signalling (Laxmi et al., 2008). Thus, PIN2 localisation to the membrane in

dark-grown *spa* mutants and the *cop1-4* mutant could contribute to the growth response of the primary root in these mutants. The localisation of PIN2 could be analysed in the mutant backgrounds with GFP-PIN2 fusion proteins.

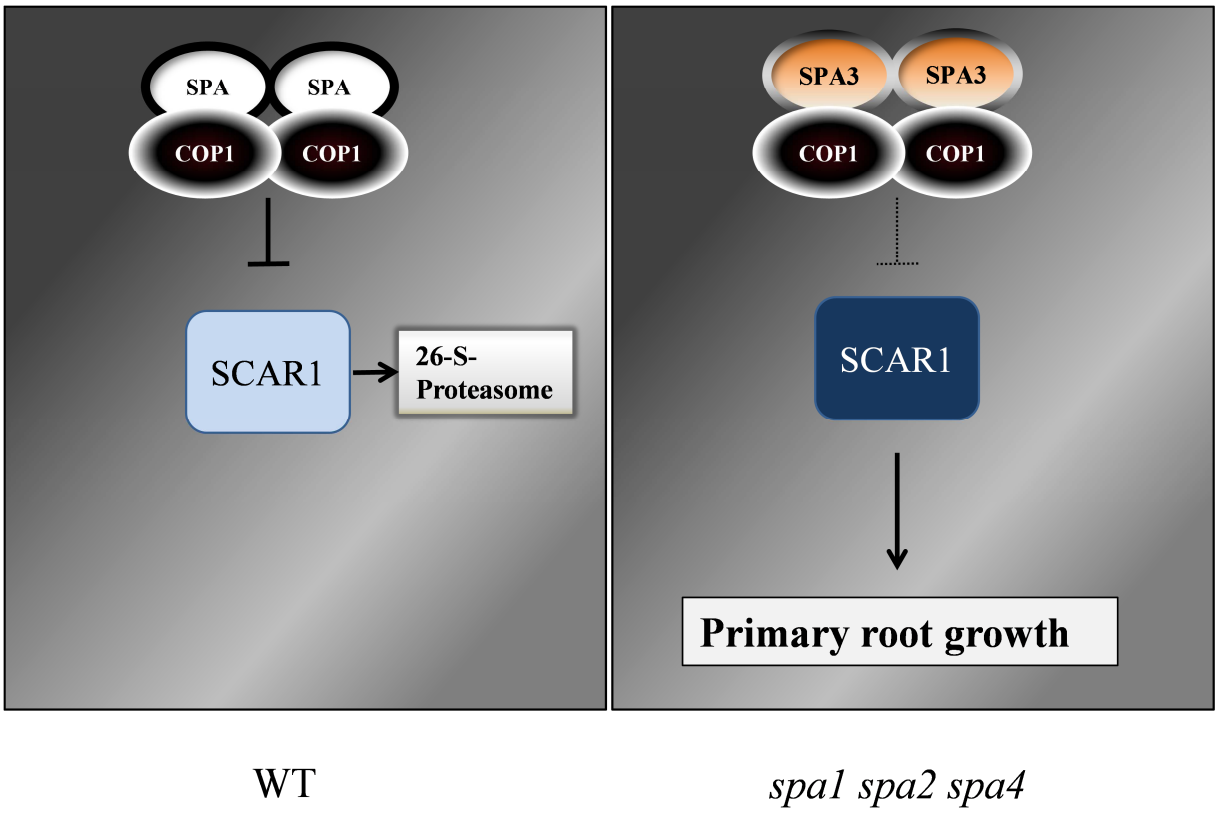

Figure III-6: Model of a possible SPA function in the root growth in darkness. SPAs could be important for the negative regulation of SCAR1 by COP1, which is important for inhibition of primary root growth in darkness (Dyachok et al., 2011).

Though commonly associated with the inhibition of auxin transport, NPA has also been shown to reduce cell proliferation in the elongation zone of roots acting by depolarising actin filaments independent of auxin (Rahman et al., 2007). Thus, it could be concluded that the *spa* triple mutant and the *cop1-4* mutant extend their roots in darkness due to cell proliferation in the root that can be abolished with NPA treatment, which may be unrelated to an auxin effect, but related to an effect on the cytoskeleton of root cells. Interestingly, COP1 was shown to interact with SCAR1 and to regulate the activity of the SCAR/WAVE protein complex, which is important for the polymerisation of actin and involved in the root elongation (Dyachok et al., 2011). COP1 and SCAR1 interact genetically in the regulation of root elongation in darkness, which firmly establishes a link between light, COP1, the SCAR/WAVE complex, the actin filaments and root elongation, which can also explain the observed phenotypes in this study obtained with NPA treatment of the roots (Fig. II-21). As *spa* triple mutant roots also kept extending in darkness, SPA proteins may contribute to the control of the

SCAR/WAVE complex in darkness (see Fig. III-6). To further investigate this possibility, all *spa* triple mutants should be tested for root growth in darkness and a physical interaction of SCAR1 with the SPA proteins should be tested. Furthermore, to support the hypothesis that *SPA* gene function in the root is necessary and sufficient to inhibit root extension in darkness, the *spa1 spa2 spa3* and *spa1 spa2 spa4* triple mutants that express SPA1 under various tissue-specific promoters could be employed to rescue the root elongation phenotype with tissue-specific expression of SPA1 (Ranja et al., 2011). If root-specific expression of SPA1 could rescue the extension phenotype, it could be hypothesised that *SPA* genes also act as regulators of the SCAR/WAVE complex in the root. If the expression of SPA1 in aerial tissues was necessary or sufficient for the rescue of the phenotype, SPA1 may likely act on the auxin transport (Salisbury et al., 2007).

Taken together, the apparent links between *SPA* gene function and auxin signalling that were further explored in this study are good starting points for further investigation of the involvement of *SPA* genes in auxin biosynthesis, transport and signalling.

III.3 The function of auxin response elements *(AuxRE)* and *G-Boxes* in auxin-induced and light-repressed promoters

Light signalling and auxin signalling are closely intertwined. Light signals manipulate the auxin system on the level of auxin biosynthesis, transport and response (Keuskamp et al., 2010; Salisbury et al., 2007; Tao et al., 2008). Light-regulated transcription factors, like PIFs and HY5 regulate the expression of auxin-induced genes and may bind to the promoter sequences to regulate their expression directly (Leivar et al., 2009; Sibout et al. 2006) On the other hand, the expression of auxin-induced genes may indirectly result from decreased auxin signalling in the light via the AuxREs without direct input from light signalling.

Six auxin-induced promoters were analysed for their light regulation (Fig. II-23). It was found that *IAA19* and *SAUR-AC1-l* were the best candidate promoters to investigate the direct input of regulation via AuxREs and G-Box motifs due to their high expression levels and robust light regulation. The light-regulation and auxin-induction was also confirmed on the transcript levels (Fig. II-24). LUC assays on the T2 generation showed that the mutation of the two G-Box elements resulted in a reduced light-repression of the *IAA19* promoter, while mutation of the four AuxREs had no effect on the light-regulation (Fig. II-25). Furthermore, the $IAA19_{mAuxRE4,3,2,1}$::LUC lines were less responsive to exogenous auxin, which indicates that the AuxRE elements are important for auxin regulation of the promoters, but not limiting for

the light-repression of the *IAA19* gene (see Fig. III-7 A for a model of *IAA19* regulation by light).

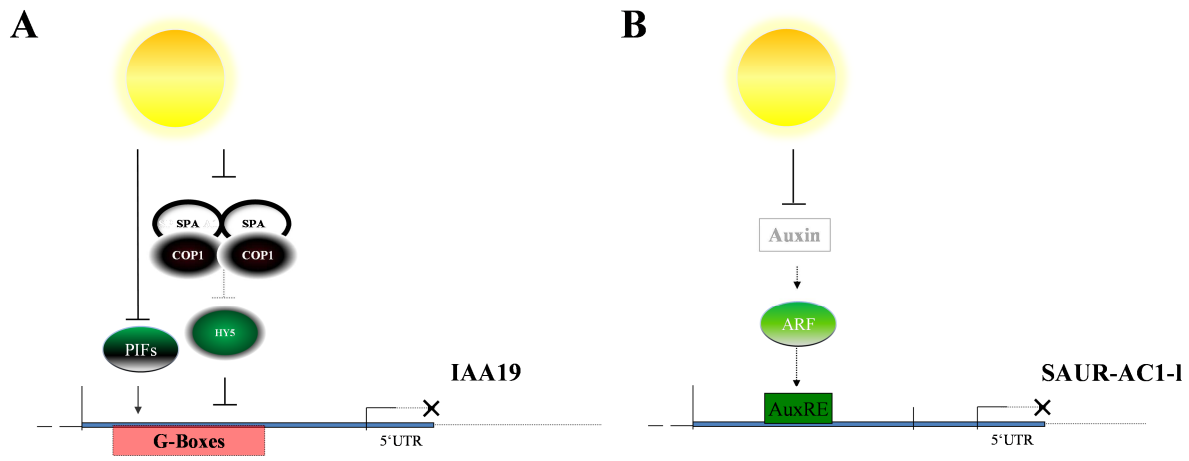

Figure III-7: Model of the regulation of *IAA19* and *SAUR-AC1-l* by light. A) The G-Boxes in the *IAA19* promoter are important for light-regulation of *IAA19* and may be bound by PIFs or HY5, which is negatively regulated by the COP1/SPA complex. **B)** The AuxRE core motif in the *SAUR-AC1-l* promoter is important for light-regulation of the gene and could be bound by ARFs in darkness, while no function was found for the G-Box.

HY5 has been implicated in the regulation of the auxin-responsiveness as mutations in the *HY5* gene cause phenotypes that are thought be represent decreased auxin sensitivity (Oyama et al., 1997). The transcript levels of *IAA19* have been reported to be up-regulated in the *hy5* and the *hy5 hyh* double mutant in Wc, which corresponds with the finding that *IAA19* transcript was lower in the *cop1-4* and the *spa1 spa3 spa4* mutants, because HY5 is repressed by the COP1/SPA complex (Fig. II-24; Seo et al., 2003; Sibout et al., 2006). Therefore, it can be hypothesised that HY5 may directly bind to the IAA19 promoter and lead to its down-regulation. Furthermore, it has been suggested that up-regulation of *IAA19* in low R:FR conditions depends on the G-Boxes in the promoter, which would suggest that PIFs also bind to the G-Boxes (Christian Fankhauser, personal communication, unpublished data). Thus, it is concluded that the G-Boxes of *IAA19* may function in a dual mode, being bound by PIF factors in darkness and in response to low R:FR conditions, which up-regulates *IAA19* and by HY5 in the light, which inhibits *IAA19* expression.

To further support the function of the G-Boxes in the regulation of the *IAA19* promoter and to show a PIF and HY5 dependency of the regulation, *IAA19::LUC*, IAA19$_{mAuxRE1,2,3,4}$::LUC and *IAA19*$_{mG\text{-}Box1,2}$*::LUC* could be crossed into the *pifq* mutant background or into PIF overexpressors, such as PIF4 or PIF5 overexpressing lines (Leivar et al., 2009; Lorrain et al., 2008). Furthermore, the promoter lines could be crossed with the *hy5* mutant and a HY5 overexpressor (Oyama et al., 1997; Hardtke et al., 2000). A direct light action on genes that

regulate the auxin response, like *Aux/IAA* genes, is furthermore in agreement with the observed difference in auxin-responsiveness of seedlings grown in darkness or shifted to R light in this study (Fig. II-20).

The *SAUR-AC1-l::LUC* promoter-reporter construct also showed a down-regulation of *SAUR-AC1-l* by light in this study. However, the mutation of the G-Box element was not sufficient to alter the light response of the promoter. In lines that expressed LUC from the promoter that contained the mAuxRE, the light-down-regulation was decreased, which was observed in two independent constructs (mAuxRE and mAuxRE/mG-Box). This indicates that the AuxRE is important for the light-regulation seen with the original promoter. The G-Box on the other hand is not contributing to this regulation (Fig. II-26). However, the $SAUR_{D1}$ promoter fragment, which only contains the G-Box motif, but not the AuxRE, also showed a strong down-regulation of the reporter gene comparable to the full length *SAUR-AC1-l* promoter. This suggests that the AuxRE is not the only factor acting on the light-regulation of the promoter and that the deleted region upstream of the $SAUR_{D1}$ promoter fragment may contain other important regulatory elements. Furthermore, the responsiveness of three independent $SAUR_{mAuxRE}$::LUC lines (T4) to exogenous auxin was similar to three SAUR::LUC lines (T4), which indicates that the AuxRE mutated was functional in the light-regulation, but not limiting for the auxin response of the promoter (Fig. II-27). It has been recognised that beside the canonical AuxRE motifs, additional cryptical AuxRE exist that confer auxin-responsiveness to promoters (Walcher and Nemhauser, 2012). Such motifs are likely to exist in the *SAUR-AC1-l* promoter, as the AuxRE element mutated was not essential for the auxin regulation of the promoter.

Taken together, the two analysed promoters that both confer down-regulation by light function differentially, as light-regulation of *IAA19* largely depends on the G-Boxes in the promoter sequence, while the AuxREs have no limiting effect on the light-regulation. On the other hand, the light-regulation of *SAUR-AC1-l* is independent on the G-Box motif, but the AuxRE motif is contributing to the regulation (see model in Fig. III-7 A,B).

The functions of the AuxREs and G-Boxes in the two promoters should be dissected with further experiments, including time-course experiments in Rc and other light conditions and may also be used to investigate the up-regulation of *IAA19* in shade conditions. Preliminary results indicated that the AuxRE motifs are not important for the upregulation of *IAA19* four hours after the onset of the low R:FR treatment, while the G-Box elements limited the response in two of three lines (data not shown).

To my knowledge, this study provides the first evidence for a direct light- regulation of an auxin-induced gene via G-Box elements from a study of promoter elements, though a large body of evidence for close light-auxin interactions has been accumulated (reviewed in Halliday et al., 2009).

IV. Materials and Methods

IV.1 Materials

IV.1.1 Plant material

The *Arabidopsis thaliana* lines created, crossed and used in this study are presented in Table 1. All plants were Col-0 ecotype except stated otherwise.

Table 1: Arabidopsis lines used in this study.
The name of mutations and transgenes, the background accession, the mutagen and references are listed.

Allele / Transgene	Mutagen	Source / Reference
cop1-4	EMS	McNellis et al., 1994
hy5-215	EMS	Oyama et al., 1997
cop1-4 hy5-215	EMS	Ulm, R., unpublished
phyB-9	EMS	Rösler et al., 2007
cop1-6	EMS	McNellis et al., 1994
cop1-6 phyB-9	EMS/T-DNA	Boccalandro et al., 2004
hfr1-101	T-DNA	Fankhauser and Chory, 2000
hfr1-101 spa1-7 spa3-1 spa4-1	T-DNA	Fackendahl, PhD Thesis, 2011
spa1-2	EMS	Hoecker et al., 1998
spa1-7	T-DNA	Fittinghoff et al., 2006
spa1-100	T-DNA	Yang et al., 2005a
spa2-1	T-DNA	Laubinger et al., 2004
spa3-1	T-DNA	Laubinger and Hoecker, 2003
spa4-1	T-DNA	Laubinger and Hoecker, 2003
spa1-7 spa2-1	T-DNA	Fittinghoff et al., 2006
spa3-1 spa4-1	T-DNA	Laubinger and Hoecker, 2003
spa2-1 spa3-1 spa4-1	T-DNA	Fittinghoff et al., 2006
spa1-7 spa3-1 spa4-1	T-DNA	Fittinghoff et al., 2006
spa1-7 spa2-1 spa4-1	T-DNA	Fackendahl, PhD Thesis, 2011
spa1-7 spa2-1 spa3-1	T-DNA	Balcerowicz et al., 2011
spa1-7 spa2-1 spa3-1 spa4-1	T-DNA	Fittinghoff et al., 2006

hy5-SALK (renamed hy5-51)	T-DNA	Ruckle et al., 2007
spa1-7 spa3-1 spa4-1 hy5-51	T-DNA	Fackendahl, PhD Thesis, 2011
phyA-211	T-DNA	Nagatani et al., 1993
spa1 spa3 spa4 phyA-211	T-DNA	Hoecker, U., unpublished
phyB-1 (RLD)	EMS	Parks et al., 2001
spa1-2 phyB-1 (RLD)	EMS	Parks et al., 2001
phyA-101 (RLD)	EMS	Dehesh et al., 1993
spa1-2 phyA-101 (RLD)	EMS	Hoecker et al., 1998
spa3 spa4 ***35S:GFP-SPA4 (FL)***	T-DNA	Fackendahl, PhD Thesis, 2011
spa3 spa4 ***35S:GFP-ΔN-SPA4***	T-DNA	Fackendahl, PhD Thesis, 2011
spa3 spa4 ***35S:GFP-Δkin-SPA4***	T-DNA	Fackendahl, PhD Thesis, 2011
spa3 spa4 ***35S:GFP-Δcc-SPA4***	T-DNA	Fackendahl, PhD Thesis, 2011
spa2 spa3 spa4 ***CAB3:GUS-SPA1***	T-DNA	Ranjan et al., 2011
spa2 spa3 spa4 ***CER6:GUS-SPA1***	T-DNA	Ranjan et al., 2011
spa2 spa3 spa4 ***KNAT1:GUS-SPA1***	T-DNA	Ranjan et al., 2011
spa2 spa3 spa4 ***ML1:GUS-SPA1***	T-DNA	Ranjan et al., 2011
spa2 spa3 spa4 ***SPA1:GUS-SPA1***	T-DNA	Ranjan et al., 2011
spa2 spa3 spa4 ***SUC2:GUS-SPA1***	T-DNA	Ranjan et al., 2011
spa2 spa3 spa4 ***TobRB7:GUS-SPA1***	T-DNA	Ranjan et al., 2011
spa1 spa2 spa3 spa4 ***SPA1::SPA1-HA***	T-DNA	Balcerowicz et al., 2011
spa1 spa2 spa3 spa4 ***SPA1::SPA2-HA***	T-DNA	Balcerowicz et al., 2011
spa1 spa2 spa3 spa4 ***SPA2::SPA1-HA***	T-DNA	Balcerowicz et al., 2011
spa1 spa2 spa3 spa4 ***SPA2::SPA2-HA***	T-DNA	Balcerowicz et al., 2011
pIAA5::LUC	T-DNA	Generated in this study
pIAA29::LUC	T-DNA	Generated in this study
pIAA30::LUC	T-DNA	Generated in this study
pIAA19::LUC	T-DNA	Generated in this study
pIAA19::GUS	T-DNA	Generated in this study
$\boldsymbol{pIAA19_{D1}}$***::LUC***	T-DNA	Generated in this study
$\boldsymbol{pIAA19_{D2}}$***::LUC***	T-DNA	Generated in this study
$\boldsymbol{pIAA19_{mAuxRE1,2,3,4}}$***::LUC***	T-DNA	Generated in this study
$\boldsymbol{pIAA19_{mG\text{-}Box1,2}}$***::LUC***	T-DNA	Generated in this study

pSAUR (AC1-l)::LUC	T-DNA	Generated in this study
pSAUR::GUS	T-DNA	Generated in this study
pSAUR$_{D1}$::LUC	T-DNA	Generated in this study
pSAUR$_{D2}$::LUC	T-DNA	Generated in this study
pSAUR$_{mAuxRE}$::LUC	T-DNA	Generated in this study
pSAUR$_{mG\text{-}Box}$::LUC	T-DNA	Generated in this study
pSAUR$_{mAuxREmG\text{-}Box}$::LUC	T-DNA	Generated in this study
DR5::GUS	T-DNA	Ulmanov et al., 1997a
spa1 spa3 spa4 ***DR5::GUS***	T-DNA	Höcker, U., unpublished
spa1 spa2 spa4 ***DR5::GUS***	T-DNA	Crossed in this study

IV.1.2 Bacterial strains

The bacterial strains used in this study were *Escherichia coli* strain *DH5α* for standard cloning procedures and the *ccdB* gene resistant *E. coli* strain *DB3.1* for handling of empty Entry Gateway™ vectors and Destination Gateway™ vectors. The respective genotypes are:

DH5α: F$^-$ Φ80dlacZΔM15 Δ(lacZYA-argF) U169 deoR recA1 endA1 hsdR17(rk$^-$, mk$^+$) phoA supE44 λ$^-$ thi-1 gyrA96 relA1

DB3.1: F$^-$ gyrA462 endA Δ(sr1-recA) mcrB mrr hsdS20 (rB$^-$,-mB$^-$) supE44 ara-14 galK2 lacY1 proA2 rpsL20 (SmR) xyl5 λ$^-$ leu mtl1

The strains were obtained from Invitrogen (Karlsruhe, Germany) and Stratagene (Santa Clara, USA). For plant transformation, *Agrobacterium tumefaciens* strain *GV3101* (pMK90RK) (Koncz *et al.*, 1994) was used.

IV.1.3 Cloning vectors

The created and used cloning vectors are listed in table 2.

Table 2: Cloning vectors used and created in this study.
The vectors are listed with name, resistance, description and reference.

Vector	Resistance	Description	Reference/Source
pDONR207	Gentamycin (Gent^r)	Gateway^{TM} entry vector, used for recombination with PCR products containing att-sites	Invitrogen
pIAA30-pDONR207		ENTRY vector carrying 2.5 kb IAA30 5' regulatory sequence	Generated in this study
pIAA5-pDONR207		ENTRY vector carrying 2.5 kb IAA5 5' regulatory sequence	
pIAA19-pDONR207		ENTRY vector carrying 2.5 kb IAA19 5' regulatory sequence	
pIAA19$_{D1}$-pDONR207		ENTRY vector carrying IAA19 5' regulatory sequence	
pIAA19$_{D2}$-pDONR207		ENTRY vector carrying IAA19 5' regulatory sequence	
pIAA19$_{mAuxRE1,2,3,4}$-pDONR207		ENTRY vector carrying mutated IAA19 5' regulatory sequence	
pIAA19$_{mAuxRE1,2,3,4mG-Box1,2}$-pDONR207		ENTRY vector carrying mutated IAA19 5' regulatory sequence	
pIAA19$_{mG-Box1,2}$-pDONR207		ENTRY vector carrying mutated IAA19 5' regulatory sequence	
pSAUR(AC1-l)-pDONR207		ENTRY vector carrying 2 kb SAUR AC1-l 5' regulatory sequence	
pSAUR$_{D1}$-pDONR207		ENTRY vector carrying SAUR 5' regulatory sequence	
pSAUR$_{D2}$-pDONR207		ENTRY vector carrying SAUR 5' regulatory sequence	
pSAUR$_{mAuxRE}$-pDONR207		ENTRY vector carrying mutated SAUR 5' regulatory sequence	
pSAUR$_{mAuxREmG-Box}$-pDONR207		ENTRY vector carrying mutated SAUR 5' regulatory sequence	
pSAUR$_{mG-Box}$-		ENTRY vector carrying mutated	

pDONR207		SAUR 5' regulatory sequence	
pGWB3	Kanamycin (Km^r), hygromycin (Hyg^r)	***GUS*** gene fusion, binary vector for plant transformation	Nakagawa et al., 2007
pGWB35		***LUC*** gene fusion, binary vector for plant transformation	
pIAA30- pGWB35		Expression of ***LUC*** driven by 2.5 kb *IAA30* regulatory sequence	Generated in this study
pIAA5-pGWB35		Expression of ***LUC*** driven by 2.5 kb *IAA5* regulatory sequence	
pIAA19-pGWB3		Expression of ***GUS*** driven by 2.5 kb *IAA19* regulatory sequence	
pIAA19-pGWB35		Expression of ***LUC*** driven by 2.5 kb *IAA19* regulatory sequence	
pIAA19$_{D1}$-pGWB35		Expression of ***LUC*** driven by *IAA19* 5' regulatory sequence	
pIAA19$_{D2}$-pGWB35		Expression of ***LUC*** driven by *IAA19* 5' regulatory sequence	
pIAA19$_{mAuxRE1,2,3,4}$-pGWB35		Expression of ***LUC*** driven by mutated *IAA19* 5' regulatory sequence	
pIAA19$_{mAuxRE1,2,3,4mG-Box1,2}$-pGWB35		Expression of ***LUC*** driven by mutated *IAA19* 5' regulatory sequence	
pIAA19$_{mG-Box1,2}$-pGWB35		Expression of ***LUC*** driven by mutated *IAA19* 5' regulatory sequence	
pSAUR(AC1-l)-pGWB3		Expression of ***GUS*** driven by 2 kb *SAUR AC1*-l 5' regulatory sequence	
pSAUR(AC1-l)-pGWB35		Expression of ***LUC*** driven by 2 kb *SAUR AC1-l* 5' regulatory sequence	
pSAUR$_{D1}$-pGWB35		Expression of ***LUC*** driven by *SAUR* 5' regulatory sequence	
pSAUR$_{D2}$-pGWB35		Expression of ***LUC*** driven by *SAUR* 5' regulatory sequence	
pSAUR$_{mAuxRE}$-pGWB35		Expression of ***LUC*** driven by mutated *SAUR* 5' regulatory sequence	

pSAUR*$_{mAuxREmG\text{-}Box}$-*pGWB35		Expression of ***LUC*** driven by mutated *SAUR* 5' regulatory sequence	
pSAUR*$_{mG\text{-}Box}$-*pGWB35		Expression of ***LUC*** driven by mutated *SAUR* 5' regulatory sequence	

IV.1.4 Oligonucleotides

Oligonucleotides were used for cloning, site-directed mutagenesis, sequencing, genotyping and qRT-PCR, respectively and are listed in Table 3. Mutagenic nucleotides are in red and capitalised.

Table 3: The oligonucleotides created and used in this study.
The oligonucleotides (primers) are listed with name, sequence and further description and reference.

Name	Sequence (5'-3')	Method/Reference
IAA30 2,5kb Prom for	ggggacaagtttgtacaaaaaagcaggctcgtattgtttttatactttcacaaatagga	GATEWAY cloning
IAA30 Prom rev	ggggaccactttgtacaagaaagctgggtctttttttatttcttttactatttctctc	GATEWAY cloning
IAA30 Prom int I for	gaaacaagttacgttgtacatataaac	Sequencing
IAA30 Prom int II for	gatgtgttttggtctctgcc	Sequencing
IAA5 prom for	ggggacaagtttgtacaaaaaagcaggctcgtgatcacttttggtttttcctatttc	GATEWAY cloning
IAA5 Prom rev	ggggaccactttgtacaagaaagctgggtgctttgatgttttgattgaaagtattg	GATEWAY cloning
IAA5 Prom int I	ctcacatcatcatggctcg	Sequencing
IAA5 Prom int II	ctattaatgatgcaacaatctgaac	Sequencing
pIAA29 prom 2.5kb for	ggggacaagtttgtacaaaaaagcaggctctcgcgggatgaagcagatac	GATEWAY cloning
pIAA29 5' rev	ggggaccactttgtacaagaaagctgggtcttctaaggcagcttcgtctttg	GATEWAY cloning
IAA29 Prom int I for	caacaccatatttttatagctttac	Sequencing
IAA29 Prom int II for	ggacgttgtccgttccaac	Sequencing
IAA19 2,5kb Prom for	ggggacaagtttgtacaaaaaagcaggctgcgagttctaaatttttgacttaactaaaag	GATEWAY cloning
IAA19 prom rev	ggggaccactttgtacaagaaagctgggtcttcttgaacttcttttttcctc	GATEWAY cloning
IAA19 Prom int I for	gactacctgaatttccagttg	Sequencing
IAA19 Prom int II for	gttcgagactaactttggagat	Sequencing
SAUR AC1-L Prom compl for	ggggacaagtttgtacaaaaaagcaggctcgagacactcctgtcttcataaac	GATEWAY cloning
SAUR AC1-L Prom rev	ggggaccactttgtacaagaaagctgggtcttcgagtattagaaagaaaaaaaac	GATEWAY cloning
19 mAuxRE1 for	gtctctgcccccactttgGctccccacacaaactgaataac	Site-directed mutagenesis
19 mAuxRE1 rev	gttattcagtttgtgtggggagCcaaagtgggggcagagac	Site-directed mutagenesis

Name	Sequence (5'-3')	Method/Reference
19 mAuxRE2 for	cctcagttgacctgGctctgccccccactttgtctcc	Site-directed mutagenesis
19 mAuxRE2 rev	ggagacaaagtgggggcagagCcaggtcaactgagg	Site-directed mutagenesis
19 mAuxRE3 for	cagcaccaaacttatgGctctcatgtgaccgacc	Site-directed mutagenesis
19 mAuxRE3 rev	ggtcggtcacatgagagCcataagtttggtgctg	Site-directed mutagenesis
19 mAuxRE4 for	cgtataagaaacatgagCcatgtcacaatcac	Site-directed mutagenesis
19 mAuxRE4 rev	gtgattgtgacatgGctcatgtttcttatacg	Site-directed mutagenesis
IAA19 mGbox 1 for	gatatcaaatgactccaATtgtcgatattgg	Site-directed mutagenesis
IAA19 mGbox 1 rev	ccaatatcgacaATtggagtcatttgatatc	Site-directed mutagenesis
IAA19 mGbox 2 for	catataatttcaATtggcccaacttg	Site-directed mutagenesis
IAA19 mGbox 2 rev	caagttgggccaATtgaaattatatg	Site-directed mutagenesis
IAA19 G1 BACK for	gatatcaaatgactccacgtgtcgatattgg	Site-directed mutagenesis
IAA19 G1 BACK rev	ccaatatcgacacgtggagtcatttgatatc	Site-directed mutagenesis
IAA19 G2 BACK for	catataatttcacgtggcccaacttg	Site-directed mutagenesis
IAA19 G2 BACK rev	caagttgggccacgtgaaattatatg	Site-directed mutagenesis
SAUR mAuxRE1 for	catcgtatttttcttgGctcttgggtagatattttc	Site-directed mutagenesis
SAUR mAuxRE1 rev	gaaaatatctacccaagagCcaagaaaaatacgatg	Site-directed mutagenesis
SAUR mGBOX1 for	gcttataatgttcaATtgtacaacgtttacgtc	Site-directed mutagenesis
SAUR mGBOX1 rev	gacgtaaacgttgtacaATtgaacattataagc	Site-directed mutagenesis
IAA19 D1 for	ggggacaagtttgtacaaaaaagcaggctgcgtcacaatca ctttaaaagttttcc	Deletion construct, GATEWAY cloning
IAA19 D2 for	ggggacaagtttgtacaaaaaagcaggctgccccacacaaa ctgaataacaag	Deletion construct, GATEWAY cloning
SAUR D1 for	ggggacaagtttgtacaaaaaagcaggctgcgggtagatatt ttcagatattttg	Deletion construct, GATEWAY cloning
SAUR D2 for	ggggacaagtttgtacaaaaaagcaggctgccccacacaaa ctgaataacaag	Deletion construct, GATEWAY cloning
PIL1f Lo08	aaattgctctcagccattcgtgg	RT-PCR; Lorrain et al., 2008
PIL1r Lo08	ttctaagtttgaggcggacgcag	RT-PCR; Lorrain et al., 2008
ATHB2r Lo08	gcatgtagaactgaggagagagc	RT-PCR; Lorrain et al., 2008
ATHB2f Lo08	gaggtagactgcgagttcttacg	RT-PCR; Lorrain et al., 2008
hfr1f Lo08	taaattggccattaccaccgttta	RT-PCR; Lorrain et al., 2008
hfr1r Lo08	accgtgaagagactgaggagaaga	RT-PCR; Lorrain et al., 2008
XTR7 f Ho09	cggcttgcacagcctctt	RT-PCR; Hornitschek et al., 2009
XTR7 r Ho09	tcggttgccacttgcaatt	RT-PCR; Hornitschek et al., 2009
SAUR AC1L_for II	acgggcggtttgagtttac	RT-PCR
SAUR AC1L_rev II	tgggattaacgaatctgagaag	RT-PCR
IAA19_for RT II	tgctaccgggtttgggctgc	RT-PCR

Name	Sequence (5'-3')	Method/Reference
IAA19_rev RT II	accagctccttgcttcttgttcaagtc	RT-PCR
TAA1 Frlin_11 F	caagaagcatgtccgagtca	RT-PCR; Franklin et al.,2011
TAA1 Frlin_11 R	agcttcatgttggcgagtct	RT-PCR; Franklin et al.,2011
YUC2 F	ataggcggtgtgggttatg	RT-PCR
YUC2 R	catccttcttccctccggtt	RT-PCR
YUC8 F	atgcccttccctgaggactt	RT-PCR
YUC8 R	gatgaactgacgcttcgtcg	RT-PCR
YUC9 F	gtcccattcgttgtggtcg	RT-PCR
YUC9 R	ttgccacagtgacgctatgc	RT-PCR
FT F_Wol08	ctcaggaacttctatactttggttatg	RT-PCR; Wollenberg et al., 2008
FT R_Wol08	gttccagttgtacgagggatatcag	RT-PCR; Wollenberg et al., 2008
CO F_Wol08	cattaaccataacgcatacatttcatc	RT-PCR; Wollenberg et al., 2008
CO F_Wol08	tccggcacaacaccagttt	RT-PCR; Wollenberg et al., 2008
FLC F_Adams09	ggatccatgggaagaaaaaaacta	RT-PCR; Adams et al., 2009
FLC R_Adams09	ggtacctcacacgaataaggtacaaagttca	RT-PCR; Adams et al., 2009
SPA1 RT F	tcttaccgatgccaatgact	RT-PCR; Maier, A. unpublished
SPA1 RT R	cacacgctcgacacacaaactg	RT-PCR; Maier, A. unpublished
SPA2 RT F	tcaggtaaggacatagaggaggac	RT-PCR; Maier, A. unpublished
SPA2 RT R	tgtagaactttgattgacccattt	RT-PCR; Maier, A. unpublished
SPA3 RT for	tcgtgtaccacaaggcattc	RT-PCR
SPA3 RT rev	tcgtgtaccacaaggcattc	RT-PCR
SPA4 RT F4	cgtgtttgtctctttatgtaatca	RT-PCR; Fackendahl, 2011
SPA4 RT R3	gaggagacagggcagaatag	RT-PCR; Fackendahl, 2011
UBQ10 F	cacactccacttggtcttgcgt	RT-PCR, Balcerowicz et al., 2011
UBQ10 R	tggtctttccggtgagagtcttca	RT-PCR, Balcerowicz et al., 2011

IV.1.5 Chemicals

Chemicals and reagents were ordered from the following companies: Applichem (Darmstadt, Germany), Applied Biosystems (Carlsbad, USA), Bio-Rad Laboratories (Hercules, USA), Clontech (Palo Alto, USA), Colgate-Palmolive (Hamburg, Germany), Difco (Detroit, USA), Duchefa (Haarlem, Netherlands), Gibco BRL (Neu Isenburg, Germany), Fermentas (St. Leon-Rot, Germany), Invitrogen (Karlsruhe, Germany), Merck (Darmstadt, Germany), Promega (Mannheim, Germany), Riedel-de-Haen (Seelze, Germany), Roche (Mannheim, Germany),

Roth (Karlsruhe, Germany), Serva (Heidelberg, Germany), Sigma-Aldrich (München, Germany), Thermo Scientific (Rockford, USA), VWR (Darmstadt, Germany).

IV.1.6 Antibiotics

Antibiotics were dissolved and stored at -20°C as sterile-filtered 1000x stock solutions (except 100x in case of spectinomycin). Used antibiotics are listed in Table 4.

Table 4: Antibiotics used for selective growth media

Antibiotics	Abbreviation	Concentration	Dissolvent (if not dH_2O)
Ampicillin	**(Amp^r)**	**100 mg/ml**	
Gentamycin	**($Gent^r$)**	**15 mg/ml**	
Hygromycin	**(Hyg^r)**	**50 mg/ml**	
Kanamycin	**(Km^r)**	**50 mg/ml**	
Rifampicin	**(Rif^r)**	**100 mg/ml**	**DMSO**
Spectinomycin	**($Spec^r$)**	**10 mg/ml**	

IV.1.7 Enzymes

All enzymes (including restriction endonucleases, polymerases, clonases and reverse transcriptase) were purchased from Fermentas (St. Leon-Rot, Germany), Clontech (Palo Alto, USA) and Invitrogen (Karlsruhe, Germany).

IV.1.8 Antibodies

Antibodies were diluted from stocks before use in TBS buffer (NaCl 0.14 M; Tris 10 mM; pH 7.3 (HCl)) containing 4% (w/v) non-fat milk powder (Table 5). Secondary antibodies were conjugated to the horse radish peroxidase (HRP).

Table 5: Primary and secondary antibodies for immunoblot analysis

Primary antibodies		
Antigen	**Dilution**	**Reference / Supplier**
α-SPA1 (rabbit)	1:300	Maier, A., PhD Thesis, 2011
α-Tubulin (mouse)	1:50000	Sigma-Aldrich (München, Germany)
Secondary antibodies		
Antigen	**Dilution**	**Reference / Supplier**
α-mouse (goat)	1:10000	Sigma-Aldrich (München, Germany); HRP-conjugated
α-rabbit (goat)	1:80000	Sigma-Aldrich (München, Germany); HRP-conjugated

IV.1.9 Media

Media for bacteria and plant growth were prepared as listed below:

Luria Bertani (LB) medium

Tryptone 10.0 g/l

Yeast extracts 5.0 g/l

NaCl 5.0 g/l

1.5% (w/v) agar was added for LB plates

Murashige and Skoog (MS) medium for plants

MS salt 4.62 g/l

pH 5.8

1 % (w/v) agar was added for MS plates

Black MS medium (for shade avoidance experiments)

MS salt 4.62 g/l

Charcoal 10 g/l

pH 5.8

1 % (w/v) agar was added for black MS plates

All media were autoclaved at 121°C for 20 min.

IV.2 Methods

IV.2.1 Molecular biological methods

Precipitation of DNA and RNA, gel electrophoresis, staining of DNA and other standard methods were performed according to standard protocols (Sambrook and Russell, 2001). Purification of nucleic acids was performed with the *Qiagen Gel Extraction Kit* or *Qiagen PCR Purification Kit* (Qiagen GmbH, Hilden, Germany). Plasmids were purified from *E.coli* with the *Qia-prep Spin Miniprep Kit* or *Qia-prep Vacuum Miniprep Kit* (Qiagen).

IV.2.1.1 Polymerase chain-reaction (PCR)

PCRs were performed with 100 ng of genomic DNA from plants, 1 µl cDNA or 100 ng of plasmid DNA as template in a volume of 20 µl when using Taq polymerase and 50 µl for Pfu polymerase protocol (Oligonucleotides 0.2 µM; dNTPs 0.5 mM; 1x PCR reaction buffer).
For a standard reaction 1 µl of Taq polymerase was used.
Standard PCR runs consisted of a first step of denaturation by 95°C for 5 min followed by 35 to 40 cycles of denaturing at 95°C for 30 sec, annealing at 56°C for 30 sec and elongation at 72°C for 1 min / 1 kb. Final elongation step was 5 min.

IV.2.1.2 PCR based site-directed mutagenesis

Site-directed mutagenesis was performed according to the *Stratagene kit* protocol (www.stratagene.com). PCR was run using Pfu proof-reading polymerase (5 µl 10X Pfu buffer; 1µl 10 mM dNTPs; 2µl of each primer (100 nM); 1 µl template vector (50 ng/µl); 1 µl Pfu DNA polymerase; ad to 50 µl dH_2O) amplifying the vector carrying the target sequence with two specific primers containing the desired point-mutation (Primers designed with Primer3, http://biotools.umassmed.edu/ bioapps/primer3_www.cgi). The PCR reaction was performed with the following steps: 1. 95°C for 1 minute 2. 95°C for 30 seconds, 60°C for 30 seconds, followed by 72°C for 1 minute/kb of plasmid with 18 cycles and a final extension with 72°C degrees for 10 minutes.
The template vector was subsequently digested by DpnI for 2h at 37°C and the mix transformed into chemically competent DH5-α. Vector DNA from clones was test-digested and sequenced to obtain clones carrying the desired mutation.

IV.2.1.3 Molecular cloning Gateway™ technology

BP and LR recombination reactions were performed as described in the manufacturer's manual (Invitrogen, Karlsruhe, Germany). The pDONR207 (Invitrogen, see Table 2) was used as Entry Gateway™ vector for all BP reactions performed in this thesis.

IV.2.1.4 Transformation of *E.coli* cells

Cells of the *E.coli* strains *DH5α* and *DB3.1* were made chemically competent, flash frozen and stored at -80°C (Inoue *et al.*,1990).
For transformation, a test tube with 50 µl suspension of the competent cells was placed on ice and incubated with 10 to 100 ng of plasmid DNA for 15 min. After incubation at 42°C for 1

½ min ("heat-shock") the tube was placed on ice for 1 min. 500 µl liquid LB medium was added to the mixture and incubated at 37°C for 45 min. The transformation suspension was centrifuged for 30 sec at 14000 rpm and the supernatant removed up to approximately 50 µl medium. The pellet was resuspended and the suspension plated onto LB plates containing antibiotics.

IV.2.1.5 Transformation of *A.tumefaciens* cells

Electro-competent cells of *A. tumefaciens* strain GV3101 (pMK90RK) were transformed with approximately 100 ng of vector DNA employing the *MicroPulser*™ electroporator (Bio-Rad laboratories, Hercules, USA) according to the manufacturer's manual. 500 µl liquid LB medium was immediately added to the cuvette after the current surge and the suspension incubated at 37°C for 45 min. The transformation suspension was centrifuged for 30 sec at 14000 rpm. Transformed cells were resuspended in 50 µl LB medium and plated onto selective media.

IV.2.1.6 DNA Sequencing

DNA sequences were verified by sequencing, which was undertaken by GATC (Konstanz, Germany). The quality of the sequencing result was examined with *4Peaks* software (Mekentosj B.V., Amsterdam, The Netherlands)

IV.2.1.7 DNA sequence management

Sequence data was analysed, edited and stored using *Vector NTI*® (Invitrogen) and *Lasergene*® (DNASTAR, Madison, USA) software packages.

IV.2.2 Transcript analysis

IV.2.2.1 Extraction of total plant RNA

Total RNA from Arabidopsis seedlings was obtained with the *RNeasy Plant Mini Kit* (Qiagen, Hilden, Germany) according to the manufacturer's manual for plant tissue. The concentration of the total RNA was determined in 1.5 µl of the extract using a Nanodrop® spectrophotometer (Thermo Scientific). The integrity of the total RNA was analysed on a 2% agarose gel, checking for the visibility of the characteristic rRNA bands.

IV.2.2.2 Reverse transcription of plant mRNA

1 µg of total RNA was DNase treated (2 µl DNase (RNase-free); 2 µl of 10x DNase buffer (Fermentas (St. Leon-Rot, Germany); ddH2O (RNase-free)) for 1 h at 37°C.
2 µl of EDTA (25mM) was added and the DNase digest incubated at 65°C for 10 min. Oligo-$(dT)_{18}$ primers were added to the digested RNA and denatured at 72°C for 10 min in a PCR cycler. The PCR reaction tube was afterwards directly placed on ice and the reverse transcriptase mix (4 µl of 5 mM dNTPs; 8 µl of 5x reverse transcriptase buffer; 1 µl of RevertAID^{TM} H Minus M-MuLV reverse transcriptase (Fermentas)) was added. The sample was incubated for 1 h at 42°C and finally at 70°C for 10 min in a PCR cycler. The obtained cDNA was stored at -20°C.

IV.2.2.3 Quantitative RT-PCR-PCR (qRT-PCR)

1 µl of cDNA was used as template in a 25 µl qRT-PCR reaction (12,5 µl *POWER SYBR Green PCR mix* (Applied Biosystems, Darmstadt, Germany); 0,25 µl of each gene specific primer (100 nM); 11 µl of autoclaved ddH_2O). The qRT-PCR was performed and analysed by the *7300 Real-Time PCR System* (Applied Biosystems). Two to three biological replicates were used and each was analysed in technical duplicates. C_t values gained from the detection were statistically evaluated using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001). *UBQ10* was amplified as endogenous control.

IV.2.3 Biochemical methods

IV.2.3.1 Protein extraction and preparation

A sample of around 200 mg of seedlings was harvested and flash frozen in liquid nitrogen. The tissue was subsequently ground with cooled mortar and pistil and the resulting powder resuspended in 150 µl protein extraction buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA, 10% glycerol, 1 mM DTT, 1% protease inhibitor cocktail (Sigma), 10 µM MG132, 1% Triton-X-100) per 100 mg tissue. The lysate was centrifuged at 4°C for 15 min at 14000 rpm and the supernatant transferred to a new reaction tube. The protein concentration was determined with the Bradford assay (Bio-Rad) using a 1:10 dilution of protein extract. 5x Laemmli buffer (310 mM Tris-HCl pH 6,8, 10% (w/v) SDS, 50% (v/v) Glycerol; 0,5% (w/v) Brom phenol blue, 500 mM DTT) (Laemmli *et al.*, 1970) was added to the protein extract and

the mixture was incubated at 96°C for 5 min. The obtained protein samples were stored at -20°C.

IV.2.3.2 SDS polyacrylamide gel electrophoresis (SDS-PAGE)

Discontinuous SDS polyacrylamide gels were prepared according to Laemmli (Laemmli 1970). The stacking gel contained 5% acrylamide, while the separation gel contained 7,5% acrylamide. Protein samples of similar total protein amount were loaded onto the gel (40µg) and separated. The proteins were then transferred onto an activated PVDF membrane (GE Healthcare, Piscataway, USA) using a *semidry blotting system* (LTF, Germany), employing *Towbin* buffer (96 mM Glycin, 10 mM Tris, 10% (v/v) Methanol). The transfer was achieved with a current of 0.35 mA / cm^2 for 2 h.

IV.2.3.3 Immunoblot analysis

The PVDF membrane was blocked using *Roti®-Block* (Roth, Karlsruhe, Germany) and incubated in the primary antibody for 2 h at room temperature or over-night at 4°C. After washing three times for 5 min with TBS-T buffer (NaCl 0.14 M; Tris 10 mM; Tween® 20 0.1 % (v/v) pH 7.3), the membrane was incubated with the corresponding secondary antibody conjugated to a horseradish peroxidase (HRP) for 1 h. After washing three times for 5 min again, bioluminescence was triggered with the *ECL Plus™ Western Blotting Detection Reagents* (GE Healthcare, Piscataway, USA) or *SuperSignal® West Femto Maximum Sensitivity Substrate kit* (Thermo Scientific, Rockford, USA) according to the manufacturers' manuals and detected with a LAS-4000 mini (Fujifilm, www.fujifilm.com). Intensities of specific protein bands were quantified with *Multi-Gauge 4.0* software (Fujifilm) and normalized to tubulin (TUB) signals.

IV.2.4 Plant growth and transformation

IV.2.4.1 Seed sterilisation

Seeds were surface-sterilized by incubation in a chlorine gas atmosphere (80 ml of sodium hypochlorite; 2.5 ml of concentrated hydrochloric acid) for approximately 3 hours. Seeds were subsequently transferred to a sterile bench and incubated for 1 h to let the gas evaporate.

IV.2.4.2 Plant growth

Seeds that were to be used in the same experiment and that originated from different seed batches were regrown in the green-house and harvested at the same point in time for synchronization.

Seeds for seedling analysis were sterilized (see previous section), sown on sterile MS plates or dropped into liquid MS (+ 1% sucrose) and incubated at 4°C for three to four days. After 3 h of white light treatment at 21°C, the plates were either kept in white light (Wc) or moved back to darkness for 21 h at 21°C and subsequently incubated in the desired light regime at 21°C. Monochromatic light was produced by LED light sources (Quantum Devices, Barneveld, WI, USA). Prior of being sown on soil, Arabidopsis seeds were incubated at 4°C in darkness for three to four days in water. Seeds were sown in a mixture of three parts soil and one part vermiculite. Plants were either grown in the greenhouse for propagation or in light chambers for plant analysis (Percival Scientific).

IV.2.4.3 *A.tumefaciens*-mediated stable transformation of Arabidopsis

The floral dip method was applied for Agrobacterium-mediated transformation of Arabidopsis flowering organs (Clough and Bent, 1998).

IV.2.5 Strategy of transgenic plant generation for promoter analysis

The 2.5 kb 5' upstream genomic regions including the 5'UTRs (or the complete 5' upstream region up to the next gene) of *AUX/IAA5, 19, 29, 30* and *SAUR AC1-l* were amplified from genomic DNA and cloned into pDONR207 (Invitrogen, gent^r) by BP reaction, resulting in promoter entry clones verified by sequencing and restriction analysis. They were recombined by LR clonase reaction into pGWB35 and pGWB3, providing *LUC* and *GUS* gene fusions for promoter analysis (Nakagawa et al., 2007). The resulting vectors are listed in table 2 and represented by vector maps in figure IV-1. Primers used for the amplification of the promoters are listed in table 3. The *IAA5* promoter fragment (around 2.5 kb up to the next gene) was amplified with the *IAA5 prom for / IAA5 prom rev* primers. The *IAA19* promoter was amplified with the *IAA19 2.5kb prom for / IAA19 prom rev* primers and *IAA29* with the p*IAA29 prom 2.5kb for* / *IAA29 5' rev* primers. The IAA30 promoter fragment was amplified with the IAA30 2.5 kb prom for / IAA30 prom rev primers and the complete 5' SAUR promoter fragment was amplified with the *SAUR AC1-l prom compl for* / *SAUR AC1-l prom rev* primer pair.

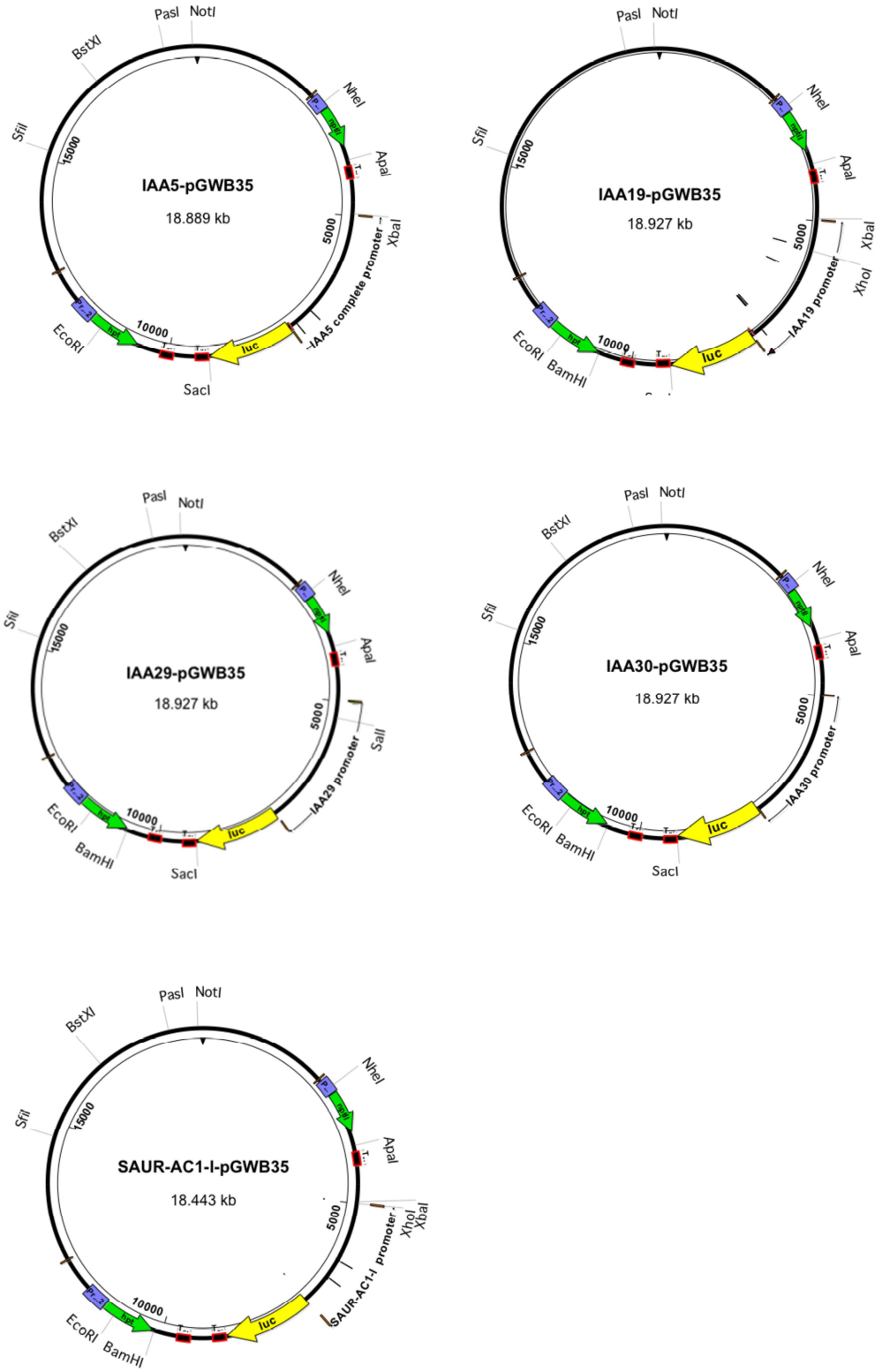

Figure IV-1: Promoter::LUC constructs. The Luciferase gene (LUC) is depicted in yellow and the insertion-site of the promoter sequence indicated. The pGWB35 carries a kanamycin resistance and a hygromycin resistance (in green). Unique restriction sites are shown.

The deletion constructs of *pIAA19* and *pSAUR AC1-l*, *pIAA19*$_{D1/D2}$ and *pSAUR AC1-l*$_{D1/D2}$ were amplified from promoter entry vectors by PCR and processed for *LUC* fusion as described for the full length promoters. For the *IAA19*$_{D1}$ and *IAA19*$_{D2}$ fragments, the primers *IAA19 D1 for* or *IAA19 D2 for* were combined with the *IAA19 prom rev* primer. Similarly, for the *SAUR AC1-l*$_{D1}$ and *SAUR AC1-l*$_{D2}$ fragments, the primers *SAUR D1 for* and *SAUR D2 for* were used with the *SAUR AC1-l prom rev* primer.

Point mutated versions of *IAA19* and *SAUR AC1-l* promoters were generated by site-directed mutagenesis (See IV.2.1.2) of the two promoter entry vectors pIAA19-pDONR207 and pSAUR-pDONR207. Mutations were generated in core *AuxRE* and *G-Box* sequences and verified by sequencing and test digestion.

The *IAA19* promoter sequence was mutated in the G-Boxes in two consecutive PCR reactions using the primer pairs *IAA19 mGbox 1 for* / *IAA19 mGbox 1 rev* and *IAA19 mGbox 2 for* / *IAA19 mGbox 2 rev*. The *IAA19*$_{mAuxRE1,2,3,4}$ plasmid was generated from a previously obtained *IAA19*$_{mG\text{-}Box1,2mAuxRE1,2,3,4}$ plasmid by mutation of the mutated *G-Boxes* back to the Col-0 sequence. The four primer pairs for the consecutive mutation of the four AuxREs were *19mAuxRE1 for/rev to 19AuxRE4 for/rev*. The G-Boxes were mutated back the original sequence by the two primer pairs *IAA19 G1 BACK for/rev* and *IAA19 G2 BACK for/rev* in consecutive PCR based site directed mutagenesis applications. The SAUR promoter sequence was mutated with the primer pair *SAUR mAuxRE1 for/rev* and the *SAUR mGBOX1 for/rev* primers, respectively. For details, refer to the supplemental figures S6 and S7, which display detailed sequence maps of the two promoter fragments.

Successfully mutated promoters were recombined with pGWB35 (::*LUC*) as described before. Transgenic plants carrying *Promoter::LUC* and *Promoter::GUS* fusions of *SAUR AC1-l*, *IAA5*, *IAA19*, *IAA29* and *IAA30* promoter sequences and of mutated or deleted versions were generated by the floral dip method and the T1 generation was harvested and selected on MS plates containing kanamycin. Resistant plants were propagated in the green house to obtain the T2 seeds. Around 20 T2 lines were screened for total Luciferase activity and the light regulation (24 h Rc/darkness). Segregation on kanamycin plates was analysed and homozygous T3 lines generated and propagated to T4 where indicated.

IV.2.6 Crossing of plant lines

The *DR5::GUS* line was crossed into the *spa1-7 spa2-1 spa4-1* mutant background (Ulmasov et al., 1997 a; Fackendahl, unpublished). The F1 generations from two independent crosses

were propagated and the obtained F2 generation screened for plants exhibiting a *spa1 spa2 spa4* phenotype in darkness with *spa1 spa2 spa4*, *spa1 spa2* and Col-0 WT as controls.
The F3 seedlings were selected for a homozygous *spa1 spa2 spa4* mutant phenotype in darkness again, were shifted to MS+1% sucrose plates for recreation and finally shifted to the greenhouse on soil. In parallel, around 20 F3 seedlings were treated with 10^{-5} NAA for 24 h and subsequent stained with GUS solution to confirm the DR5::GUS insertion. The coupling of the *spa1 spa2 spa4* phenotype and the DR5::GUS insertion was very rare. Two lines, **#1.3** and **#41.11** could be identified and were propagated to obtain F4 seeds.

IV.2.7 Shade avoidance setup

For adult plant growth analysis and determination of flowering time, seeds were pre-treated in water for 3 days at 4°C and plated on soil in single wells of 77-well trays in a randomized fashion. Plants were grown in constant white light at 21°C, 60% humidity for four days and were subsequently incubated in continuous low R:FR conditions in the upper shelf or kept in continuous white light on the lower shelf (Growth chamber *AR-36L;* cool-white fluorescent light sources; Percival-Scientific, Perry, USA). The upper shelf was additionally equipped with LED light sources (Quantum Devices, Barneveld, WI, USA) for far-red light emission. The white light photon fluence rate was kept constant at 50 µmol × m^{-2} × sec^{-1} in both shelves and the R:FR ratio was adjusted to 0.15 for low R:FR in the upper shelf. The R:FR ratio was 9.8 in the continuous white light conditions (lower shelf). The settings were: Upper shelf (Wc+FRc): 98% Wc and 98% far-red light LEDs (additional far-red light fluence rate: 90 µmol × m^{-2} × sec^{-1}); lower shelf (Wc): 50% Wc.
For seedling experiments and all transcript determinations and protein extractions, seeds were surface-sterilized and sown on MS plates containing 1% activated charcoal (black MS). Seeds were stratified at 4°C for three days in the dark and incubated in continuous white light (50 µmol × m^{-2} × sec^{-1}) provided by white light LED light sources. Seedlings were grown at constant 21°C. The R:FR ratio of the white light was 10.3 (Percival light chamber E-30B equipped with flora LEDs, CLF, Plant Climatics GmbH, Germany). Shade conditions were simulated by additional far-red light emitted by LED light sources in a chamber of identical construction (Model: E-30B with floral LEDs, CLF, Plant Climatics GmbH, Germany). The PAR was kept at 50 µmol × m^{-2} × sec^{-1} and the R:FR ratio adjusted to 0.23 with additional 70 µmol × m^{-2} × sec^{-1} of far-red light. The settings for the white light and low R:FR light conditions were: Wc channel: 93%; FR channel 0% (Wc) / 80% (Wc+FRc).

All photon fluence rates and ratios were quantified using a *SpectroSense2+* (Skye Instruments, Powys, United Kingdom) equipped with a 1- channel white light sensor or a 4-channel sensor (red and far-red light specific sensors) .

Furthermore, the spectral composition of the Wc and Wc+FRc conditions are shown in figure IV-1 (analysed with spectrometer F600, Stellar Net).

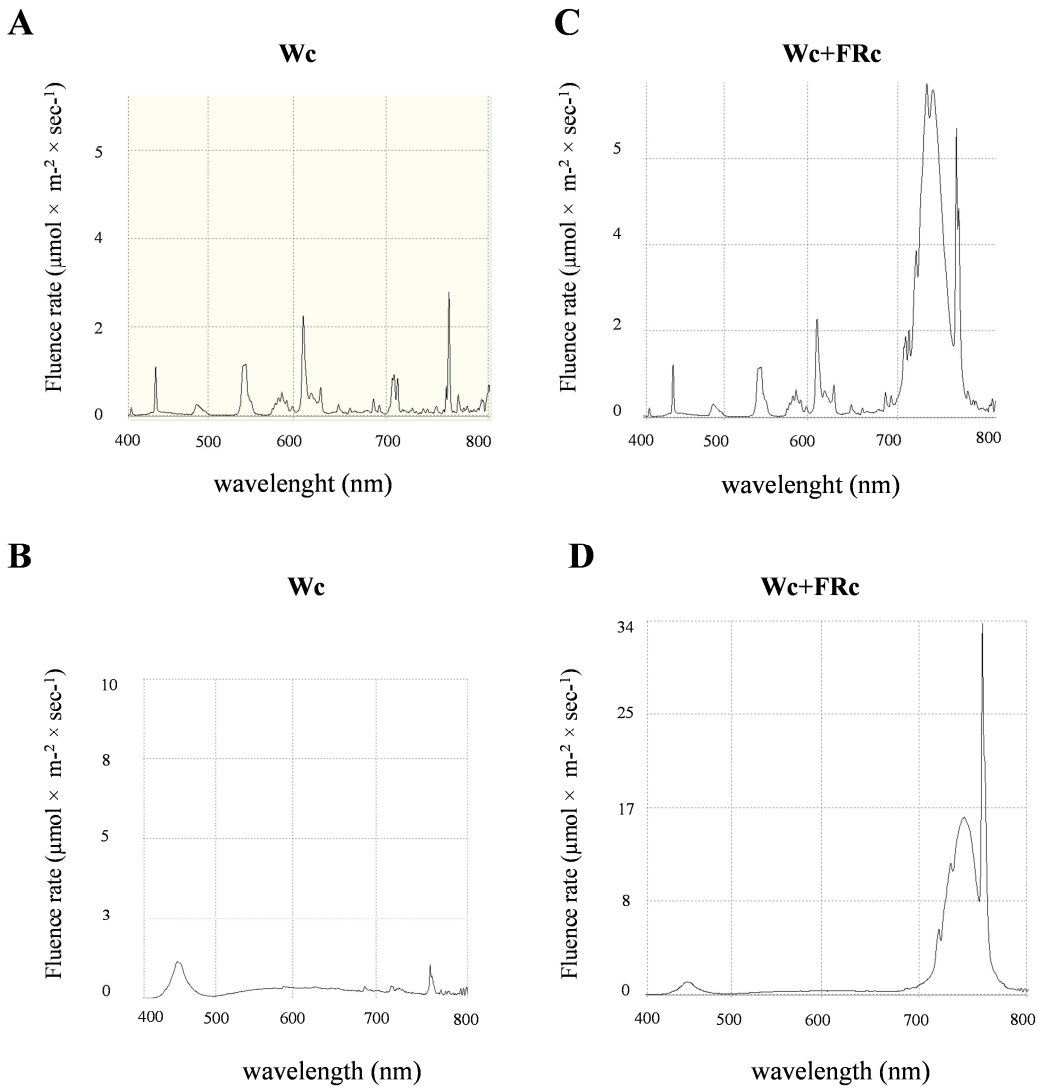

Figure IV-2: Analysis of spectral composition of the Wc and Wc+FRc conditions for seedling and adult plant growth. The fluences at the wavelengths between 400 nm and 800 nm were plotted. **A+C)** Composition of the Wc and Wc+FRc conditions used for adult plant growth. **B+D)** Composition of the Wc and Wc+FRc conditions used for seedling experiments on black MS plates.

IV.2.8 Plant phenotypic analyses

IV.2.8.1 Hypocotyl, petiole and leaf length measurements

Seedlings were flattened to the agar plates and pictures were taken with a NIKON D5000 digital camera (Nikon, www.nikon.com). Adult plant stages were documented from above or from one side. The measurements of hypocotyl length, cotyledon dimensions and leaf size were carried out with *ImageJ 1.43u* software (Wayne Rasband National Institutes of Health, USA). The values were statistically processed with *Excel 2010* (Microsoft Corporation, USA)

IV.2.8.2 Determination of flowering time

Plants were grown randomized on soil in single wells at constant distances. Flowering time in simulated shade (Wc+FRc) and continuous white light (Wc) conditions was determined by the number of true leaves at the day the first inflorescence was visible to the unaided eye and the number of days to flower from the day of sowing. At least eight plants were analysed for each genotype. The data was analysed with *Excel 2010*. The experiment was rerun twice with similar results.

IV.2.8.3 Determination of root length

Plants were grown on square MS plates incubated vertically. The root length was then determined as described in section hypocotyl length (IV.2.8.1).

IV.2.9 Quantitative luciferase assays

Around 100 mg of seedlings were harvested into a 2 ml safe-lock reaction tube that contained five metal beats (Biorad). The tissue was flash frozen in liquid nitrogen. Homogenisation of the cooled sample was achieved by shaking for 1 min at 30 sec^{-1} with a Retsch Mill®. 200 µl of extraction buffer (100 mM $NaPO_4$ pH7.5; 1 mM DTT) were added to the homogenized tissue and the tube placed on ice. After centrifugation at 4°C for 10 min at 14000 rpm, 100 µl of the supernatant was transferred to a new reaction tube.

The amount of total protein was estimated by processing 12.5 µl of a 1:10 dilution of the supernatant with the *Pierce® BCA Protein Assay Kit* (Thermo Scientific) according to the user manual (microtiter plate application). The measurements were carried out by a *Tecan M200* plate reader (Tecan, Männedorf, Schweiz).

10 µl of undiluted protein extract was added to 15 µl 2 mM EDTA in a well of a microtiter plate (Greiner Bio-one, Germany). The plate reader was programmed to inject 100 µl luciferase assay buffer (50 mM Tris-HCl, pH 7,8; 10 mM $MgCl_2$, 1 mM EDTA; 15 mM DTT; 1 mM ATP; 0,5 mM Luciferin (Roth)) into a well, halt for two seconds and count the number of emitted photons within the following ten seconds. All measurements were performed for two to three biological replicates (in case not stated otherwise) in technical duplicates for each sample.

IV.2.10 GUS assays

GUS enzymatic activity was quantified and visualised by the following methods.

IV.2.10.1 Quantitative GUS assay (MUG assay)

Protein extraction and determination of the total protein concentration was performed as described in the *quantitatve LUC assay* section (see IV.2.9). 25 µl of the undiluted protein extract were transferred to a white flat bottomed 96-well microtiter (Greiner Bio-One, Frickenhausen, Germany) plate and 100 µl qGUS assay buffer (extraction buffer; 1mM MUG (4-methylumbelliferyl-β-D-glucuronic acid)) were added. The detection of the 4-MU production was performed by a preheated *Tecan M200* plate reader (Tecan, Männedorf, Switzerland) measuring the fluorescence at 455 nm after excitation with 365 nm in each well. Data were collected 150 times at two-minute intervals at continuous 37°C and the results plotted as a time chart. The linear slop of each curve was extracted from the data and the GUS activity calculated in pmol (4-MU) × min^{-1} × ug (total protein)$^{-1}$ using a standard curve with increasing 4-MU concentrations.

IV.2.10.2 Histochemical GUS assay

GUS activity was made visible as described previously with minor modifications (Jefferson *et al.*, 1987). Seedlings or leaves were incubated in staining buffer (0.1% TritonX-100, 10 mM EDTA, pH 7; 0.5 mM $NaPO_4$, pH 7.0; 0.5 mM $K_4Fe(CN)_6$; 0.5 mM $K_3Fe(CN)_6$; 1 mM 5-bromo-4-chloro-3-indolyl-β-d-glucuronic acid (X-Gluc; Duchefa) for two to 16 hours at 37°C. The reaction was stopped by adding 70% ethanol to destain the samples. Blue staining was observed and documented with a Nikon 5000 camera.

IV.2.11 Auxin (1-naphthaleneacetic acid (NAA)) treatments

Seeds were incubated over night at -80°C to prevent fungal growth and subsequently surface-sterilized as described (see IV.2.4.1). Seedlings were grown in liquid MS supplied with 1% sucrose under white light conditions or in darkness for five days after 3h of white light treatment to synchronize germination. Sterile NAA solution or mock solution (containing DMSO) was added to the MS medium and plants were incubated for 24 h or the indicated time. Seedlings were then removed from the liquid medium, dried on a paper towel and flash frozen in liquid nitrogen. Tissue was stored at -80°C until use. Triplicates of each genotype and condition were taken as biological replicates and processed in parallel.

IV.2.12 1-N-naphthylphtalamic acid (NPA) treatments (auxin transport inhibitor)

In order to inhibit polar auxin transport, seeds were treated as described previously (IV.2.4.1) and subsequently sowed on plates that contained 5µM NPA or seedlings were transferred to NPA plates after growth on MS plates.

V. References

Abel, S., Oeller, P.W. & Theologis, 1994. Early auxin-induced genes encode short-lived nuclear proteins. *Proceedings of the National Academy of Sciences*, 91(1), 326–330.

Adams, S., Allen, T. & Whitelam, Garry C., 2009. Interaction between the light quality and flowering time pathways in Arabidopsis. *The Plant Journal*, 60(2), 257–267.

Atchley, W.R. & Fitch, W.M., 1997. A natural classification of the basic helix–loop–helix class of transcription factors. *Proceedings of the National Academy of Sciences*, 94(10), 5172 –5176.

Balcerowicz, M. et al., 2011. Light exposure of Arabidopsis seedlings causes rapid de-stabilization as well as selective post-translational inactivation of the repressor of photomorphogenesis SPA2. *The Plant Journal*, 65(5), 712–723.

Ballaré, C.L., 1999. Keeping up with the neighbours: phytochrome sensing and other signalling mechanisms. *Trends in Plant Science*, 4(3), 97–102.

Ballesteros, M.L., 2001. LAF1, a MYB transcription activator for phytochrome A signaling. *Genes & Development*, 15(19), 2613–2625.

Bauer, D. et al., 2004. Constitutive Photomorphogenesis 1 and Multiple Photoreceptors Control Degradation of Phytochrome Interacting Factor 3, a Transcription Factor Required for Light Signaling in Arabidopsis. *The Plant Cell* , 16(6), 1433 –1445.

Bennett, M.J. et al., 1996. Arabidopsis AUX1 Gene: A Permease-Like Regulator of Root Gravitropism. *Science*, 273(5277), 948–950.

Boccalandro, H.E. et al., 2005. Promotion of photomorphogenesis by COP1. *Plant Molecular Biology*, 56(6), 905–915.

Botto, J. F. & Smith, H., 2002. Differential genetic variation in adaptive strategies to a common environmental signal in Arabidopsis accessions: phytochrome-mediated shade avoidance. *Plant, Cell & Environment*, 25(1), 53–63.

Botto, Javier F. et al., 1996. Phytochrome A Mediates the Promotion of Seed Germination by Very Low Fluences of Light and Canopy Shade Light in Arabidopsis. *PLANT PHYSIOLOGY*, 110, 439–444.

Briggs, W.R. & Christie, John M., 2002. Phototropins 1 and 2: versatile plant blue-light receptors. *Trends in Plant Science*, 7(5), 204–210.

Brunoud, G. et al., 2012. A novel sensor to map auxin response and distribution at high spatio-temporal resolution. *Nature*, 482(7383), 103–106.

Carabelli, M. et al., 1993. The Arabidopsis ATHB-2 and -4 genes are strongly induced by far-red-rich light. *The Plant Journal*, 4(3), 469–479.

Carabelli, M. et al., 1996. Twilight-zone and canopy shade induction of the ATHB-2 homeobox gene in green plants. *Developmental Biology*, 93, 3530–3535.

Celenza, J.L., Grisafi, P.L. & Fink, G.R., 1995. A pathway for lateral root formation in *Arabidopsis thaliana*. *Genes & Development*, 9(17), 2131–2142.

Cerdán, P.D. & Chory, J., 2003. Regulation of flowering time by light quality. *Nature*, 423(6942), 881–885.

Chen, M., Chory, J. & Fankhauser, C., 2004. Light Signal Transduction in Higher Plants. *Annual Review of Genetics*, 38(1), 87–117.

Cheng, Y., 2006. Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in Arabidopsis. *Genes & Development*, 20(13), 1790–1799.

Cheng, Y., Dai, X. & Zhao, Y., 2007. Auxin Synthesized by the YUCCA Flavin Monooxygenases Is Essential for Embryogenesis and Leaf Formation in Arabidopsis. *THE PLANT CELL*, 19(8), 2430–2439.

Christensen, S.K. et al., 2000. Regulation of Auxin Response by the Protein Kinase PINOID. *Cell*, 100(4), 469–478.

Christie, J. M. et al., 2012. Plant UVR8 Photoreceptor Senses UV-B by Tryptophan-Mediated Disruption of Cross-Dimer Salt Bridges. *Science*, 335(6075), 1492–1496.

Clack, T., Mathews, S. & Sharrock, Robert A., 1994. The phytochrome apoprotein family inArabidopsis is encoded by five genes: the sequences and expression ofPHYD andPHYE. *Plant Molecular Biology*, 25(3), 413–427.

Cluis, C.P., Mouchel, C.F. & Hardtke, C.S., 2004. The Arabidopsis transcription factor HY5 integrates light and hormone signaling pathways. *The Plant Journal*, 38(2), 332–347.

Cole, M. et al., 2009. DORNROSCHEN is a direct target of the auxin response factor MONOPTEROS in the Arabidopsis embryo. *Development*, 136(10), 1643–1651.

Corbesier, L. et al., 2007. FT Protein Movement Contributes to Long-Distance Signaling in Floral Induction of Arabidopsis. *Science*, 316(5827), 1030 –1033.

Cosgrove, D.J., 2005. Growth of the plant cell wall. *Nat Rev Mol Cell Biol*, 6(11), 850–861.

Cosgrove, D.J., 2000. Loosening of plant cell walls by expansins. *Nature*, 407(6802), 321–326.

Crocco, C.D. et al., 2010. AtBBX21 and COP1 genetically interact in the regulation of shade avoidance. *The Plant Journal*, 64(4), 551–562.

Davis, S.J., 1999. The Arabidopsis thaliana HY1 locus, required for phytochrome-chromophore biosynthesis, encodes a protein related to heme oxygenases. *Proceedings of the National Academy of Sciences*, 96(11), 6541–6546.

Dehesh K, et al., 1993. Arabidopsis HY8 locus encodes phytochrome A. *THE PLANT CELL*, 5: 1081-1088

Deng, X.-W. et al., 1992. COP1, an arabidopsis regulatory gene, encodes a protein with both a zinc-binding motif and a Gβ homologous domain. *Cell*, 71(5), 791–801.

Deng, X.W., Caspar, T. & Quail, P H, 1991. cop1: a regulatory locus involved in light-controlled development and gene expression in Arabidopsis. *Genes & Development*, 5(7), 1172 –1182.

Devlin, P. F., Yanovsky, M. & Kay, S.A., 2003. A Genomic Analysis of the Shade Avoidance Response in Arabidopsis. *PLANT PHYSIOLOGY*, 133(4), 1617–1629.

Devlin, Paul F. et al., 1999. Phytochrome D Acts in the Shade-Avoidance Syndrome in Arabidopsis by Controlling Elongation Growth and Flowering Time. *Plant Physiology*, 119(3), 909 –916.

Dharmasiri, N., Dharmasiri, S. & Estelle, M., 2005. The F-box protein TIR1 is an auxin receptor. *Nature*, 435(7041), 441–445.

Dickopf, S., 2011. Analysis of shade avoidance responses in Arabidopsis. *Master Thesis*. University of Cologne

Duek, P.D. et al., 2004. The Degradation of HFR1, a Putative bHLH Class Transcription Factor Involved in Light Signaling, Is Regulated by Phosphorylation and Requires COP1. *Current Biology*, 14(24), 2296–2301.

Duek, P.D. & Fankhauser, C., 2005. bHLH class transcription factors take centre stage in phytochrome signalling. *Trends in Plant Science*, 10(2), 51–54.

Dyachok, J. et al., 2011. SCAR Mediates Light-Induced Root Elongation in Arabidopsis through Photoreceptors and Proteasomes. *The Plant Cell*, 23(10), 3610 –3626.

Eklöf, J.M. & Brumer, H., 2010. The XTH Gene Family: An Update on Enzyme Structure, Function, and Phylogeny in Xyloglucan Remodeling. *Plant Physiology*, 153(2), 456 –466.

Endo, M. et al., 2005. Phytochrome B in the Mesophyll Delays Flowering by Suppressing FLOWERING LOCUS T Expression in Arabidopsis Vascular Bundles. *The Plant Cell*, 17(7), 1941 –1952.

Esmon, C.A., 2006. A gradient of auxin and auxin-dependent transcription precedes tropic growth responses. *Proceedings of the National Academy of Sciences*, 103(1), 236–241.

Fackendahl, P., 2011. Functional Analysis of Arabidopsis SPA Proteins in Plant Growth Control. *PhD Thesis*. University of Cologne.

Fankhauser, C. & Chory, J., 2000. RSF1, an Arabidopsis Locus Implicated in Phytochrome A Signaling. *Plant Physiology*, 124(1), 39 –46.

Filiault, D.L. & Maloof, J.N., 2012. A Genome-Wide Association Study Identifies Variants Underlying the Arabidopsis thaliana Shade Avoidance Response. *PLoS Genetics*, 8(3), e1002589.

Fittinghoff, K., 2009. Functional Analysis of the SPA Gene Family in *Arabidopsis thaliana*. *PhD Thesis*.

Fittinghoff, K. et al., 2006. Functional and expression analysis of Arabidopsis *SPA* genes during seedling photomorphogenesis and adult growth. *The Plant Journal*, 47(4), 577–590.

Franklin, K. A. et al., 2011. PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) regulates auxin biosynthesis at high temperature. *Proceedings of the National Academy of Sciences*, 108(50), 20231–20235.

Franklin, K. A. & Quail, P. H., 2009. Phytochrome functions in Arabidopsis development. *Journal of Experimental Botany*, 61(1), 11–24.

Friml, J. et al., 2004. A PINOID-Dependent Binary Switch in Apical-Basal PIN Polar Targeting Directs Auxin Efflux. *Science*, 306(5697), 862–865.

Friml, Jiri et al., 2002. Lateral relocation of auxin efflux regulator PIN3 mediates tropism in Arabidopsis. *Nature*, 415(6873), 806–809.

Galstyan, A. et al., 2011. The shade avoidance syndrome in Arabidopsis: a fundamental role for atypical basic helix-loop-helix proteins as transcriptional cofactors. *The Plant Journal*, 66(2), 258–267.

Gao, X. et al., 2008. Cell Polarity Signaling: Focus on Polar Auxin Transport. *Molecular Plant*, 1(6), 899 –909.

Gendreau, E. et al., 1997. Cellular basis of hypocotyl growth in Arabidopsis thaliana. *PLANT PHYSIOLOGY*, 114(1), 295–305.

Gray, W.M. et al., 2001. Auxin regulates SCFTIR1-dependent degradation of AUX/IAA proteins. *Nature*, 414(6861), 271–276.

Halliday, K.J., Koorneef, M. & Whitelam, G. C., 1994. Phytochrome B and at least one other phytochrome mediate the acceleration of flowering response of Arabidopsis thaliana L. to low red/far-red ratio. *PLANT PHYSIOLOGY*, 104, 1311–1315.

Halliday, K.J., Martínez-García, J.F. & Josse, E.-M., 2009b. Integration of Light and Auxin Signaling. *Cold Spring Harbor Perspectives in Biology*, 1(6).

Hao, Y. et al., 2012. Interactions between HLH and bHLH Factors Modulate Light-Regulated Plant Development. *Molecular Plant*. First published online in February 2012. doi:10.1093/mp/sss011

Hardtke, C.S. et al., 2000. HY5 stability and activity in Arabidopsis is regulated by phosphorylation in its COP1 binding domain. *EMBO J*, 19(18), 4997–5006.

Hiltbrunner, A., 2006. FHY1 and FHL Act Together to Mediate Nuclear Accumulation of the Phytochrome A Photoreceptor. *Plant and Cell Physiology*, 47(8), 1023–1034.

Hiltbrunner, Andreas et al., 2005. Nuclear Accumulation of the Phytochrome A Photoreceptor Requires FHY1. *Current Biology*, 15(23), 2125–2130.

Hoecker, U., 2005. Regulated proteolysis in light signaling. *Current Opinion in Plant Biology*, 8(5), 469–476.

Hoecker, U. et al., 2004. The photomorphogenesis-related mutant red1 is defective in CYP83B1 , a red light-induced gene encoding a cytochrome P450 required for normal auxin homeostasis. *Planta*, 219(2), 195–200.

Hoecker, U. & Quail, Peter H., 2001. The Phytochrome A-specific Signaling Intermediate SPA1 Interacts Directly with COP1, a Constitutive Repressor of Light Signaling inArabidopsis. *Journal of Biological Chemistry*, 276(41), 38173 –38178.

Hoecker, U., Tepperman, J.M. & Quail, Peter H., 1999. SPA1, a WD-Repeat Protein Specific to Phytochrome A Signal Transduction. *Science*, 284(5413), 496 –499.

Hoecker, U., Xu, Yong & Quail, P H, 1998. SPA1: A New Genetic Locus Involved in Phytochrome A-Specific Signal Transduction. *THE PLANT CELL*, 10, 19–33.

Holm, M., 2002. Two interacting bZIP proteins are direct targets of COP1-mediated control of light-dependent gene expression in Arabidopsis. *Genes & Development*, 16(10), 1247–1259.

Hornitschek, P. et al., 2009. Inhibition of the shade avoidance response by formation of non-DNA binding bHLH heterodimers. *The EMBO Journal*, 28(24), 3893–3902.

Huala, E., 1997. Arabidopsis NPH1: A Protein Kinase with a Putative Redox-Sensing Domain. *Science*, 278(5346), 2120–2123.

Hull, A.K., 2000. Arabidopsis cytochrome P450s that catalyze the first step of tryptophan-dependent indole-3-acetic acid biosynthesis. *Proceedings of the National Academy of Sciences*, 97(5), 2379–2384.

Huq, E. et al., 2004. PHYTOCHROME-INTERACTING FACTOR 1 Is a Critical bHLH Regulator of Chlorophyll Biosynthesis. *Science*, 305(5692), 1937 –1941.

Huq, E., Al-Sady, B. & Quail, Peter H., 2003. Nuclear translocation of the photoreceptor phytochrome B is necessary for its biological function in seedling photomorphogenesis. *The Plant Journal*, 35(5), 660–664.

Hyun, Y. & Lee, I., 2006. KIDARI, Encoding a Non-DNA Binding bHLH Protein, Represses Light Signal Transduction in Arabidopsis thaliana. *Plant Molecular Biology*, 61(1-2), 283–296.

Imaizumi, T. et al., 2003. FKF1 is essential for photoperiodic-specific light signalling in Arabidopsis. Nature, 426(6964), 302–306.

Izawa, T. et al., 1994. The Rice bZIP Transcriptional Activator RITA-1 Is Highly Expressed during Seed Development. *The Plant Cell* , 6(9), 1277 –1287.

Jaillais, Y. & Chory, J., 2010. Unraveling the paradoxes of plant hormone signaling integration. *Nature Structural & Molecular Biology*, 17(6), 642–645.

Jang, I.-C. et al., 2010. Arabidopsis PHYTOCHROME INTERACTING FACTOR Proteins Promote Phytochrome B Polyubiquitination by COP1 E3 Ligase in the Nucleus. *THE PLANT CELL*, 22(7), 2370–2383.

Jang, In-Cheol et al., 2005. HFR1 is targeted by COP1 E3 ligase for post-translational proteolysis during phytochrome A signaling. *Genes & Development*, 19(5), 593 –602.

Jang, S. et al., 2008. Arabidopsis COP1 shapes the temporal pattern of CO accumulation conferring a photoperiodic flowering response. *EMBO J*, 27(8), 1277–1288.

Kepinski, S. & Leyser, O., 2004. Auxin-induced SCFTIR1-Aux/IAA interaction involves stable modification of the SCFTIR1 complex. *Proceedings of the National Academy of Sciences*, 101(33), 12381–12386.

Kepinski, Stefan & Leyser, O., 2005. The Arabidopsis F-box protein TIR1 is an auxin receptor. *Nature*, 435(7041), 446–451.

Keuskamp, D. H. et al., 2010. Auxin transport through PIN-FORMED 3 (PIN3) controls shade avoidance and fitness during competition. *Proceedings of the National Academy of Sciences*, 107(52), 22740–22744.

Keuskamp, Diederik H. et al., 2011. Blue-light-mediated shade avoidance requires combined auxin and brassinosteroid action in Arabidopsis seedlings. *The Plant Journal*, 67(2), 208–217.

Kim, S.Y., Yu, X. & Michaels, S.D., 2008. Regulation of CONSTANS and FLOWERING LOCUS T Expression in Response to Changing Light Quality. *Plant Physiology*, 148(1), 269 –279.

King, J.J. et al., 1995. A Mutation Altering Auxin Homeostasis and Plant Morphology in Arabidopsis. *THE PLANT CELL*, 7(12), 2023–2037.

Kircher, S., 1999. Light quality-dependent nuclear import of the plant photoreceptors phytochrome A and B. *THE PLANT CELL*, 11(8), 1445–1456.

Knauss, S., 2003. The Auxin-induced Maize Gene ZmSAUR2 Encodes a Short-lived Nuclear Protein Expressed in Elongating Tissues. *Journal of Biological Chemistry*, 278(26), 23936–23943.

Koo, J. et al., 2007. A GUS/Luciferase Fusion Reporter for Plant Gene Trapping and for Assay of Promoter Activity with Luciferin-Dependent Control of the Reporter Protein Stability. *Plant and Cell Physiology*, 48(8), 1121–1131.

Kozuka, T. et al., 2010. Involvement of Auxin and Brassinosteroid in the Regulation of Petiole Elongation under the Shade. *Plant Physiology*, 153(4), 1608 –1618.

Kubeš, M. et al., 2012. The Arabidopsis concentration-dependent influx/efflux transporter ABCB4 regulates cellular auxin levels in the root epidermis. *The Plant Journal*, 69(4), 640–654.

Kumar, S.V. et al., 2012. Transcription factor PIF4 controls the thermosensory activation of flowering. *Nature*, advance publication.

Kunihiro, A. et al., 2011. PHYTOCHROME-INTERACTING FACTOR 4 and 5 (PIF4 and PIF5) Activate the Homeobox ATHB2 and Auxin-Inducible IAA29 Genes in the Coincidence Mechanism Underlying Photoperiodic Control of Plant Growth of Arabidopsis thaliana. *Plant and Cell Physiology*, 52(8), 1315–1329.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680–684.

Laubinger, S. et al., 2006. Arabidopsis SPA proteins regulate photoperiodic flowering and interact with the floral inducer CONSTANS to regulate its stability. *Development*, 133(16), 3213 –3222.

Laubinger, S., Fittinghoff, K. & Hoecker, U., 2004. The SPA Quartet: A Family of WD-Repeat Proteins with a Central Role in Suppression of Photomorphogenesis in Arabidopsis. *The Plant Cell* , 16(9), 2293 –2306.

Laubinger, S. & Hoecker, U., 2003. The SPA1-like proteins SPA3 and SPA4 repress photomorphogenesis in the light. *The Plant Journal*, 35(3), 373–385.

Laxmi, A. et al., 2008. Light Plays an Essential Role in Intracellular Distribution of Auxin Efflux Carrier PIN2 in Arabidopsis thaliana. *PLoS ONE*, 3(1), S.e1510.

Lee, J. et al., 2007. Analysis of Transcription Factor HY5 Genomic Binding Sites Revealed Its Hierarchical Role in Light Regulation of Development. *The Plant Cell*, 19(3), 731 –749.

Lehman, A., Black, R. & Ecker, J.R., 1996. HOOKLESS1, an Ethylene Response Gene, Is Required for Differential Cell Elongation in the Arabidopsis Hypocotyl. *Cell*, 85(2), 183–194.

Leivar, P. et al., 2009. Definition of Early Transcriptional Circuitry Involved in Light-Induced Reversal of PIF-Imposed Repression of Photomorphogenesis in Young Arabidopsis Seedlings. *The Plant Cell* , 21(11), 3535 –3553.

Leivar, P. et al., 2008. Multiple Phytochrome-Interacting bHLH Transcription Factors Repress Premature Seedling Photomorphogenesis in Darkness. *Current Biology*, 18(23), 1815–1823.

Li, L. et al., 2012. Linking photoreceptor excitation to changes in plant architecture. *Genes & Development*, 26(8), 785–790.

Li, Linchuan et al., 2007. The possible action mechanisms of indole-3-acetic acid methyl ester in Arabidopsis. *Plant Cell Reports*, 27(3), 575–584.

Lian, H.-L. et al., 2011. Blue-light-dependent interaction of cryptochrome 1 with SPA1 defines a dynamic signaling mechanism. *Genes & Development*, 25(10), 1023 –1028.

Lin, C., 2002. Blue Light Receptors and Signal Transduction. *THE PLANT CELL*, 12, 5207–5225.

Liu, Bin et al., 2011. Arabidopsis cryptochrome 1 interacts with SPA1 to suppress COP1 activity in response to blue light. *Genes & Development*.

Liu, L.-J. et al., 2008. COP1-Mediated Ubiquitination of CONSTANS Is Implicated in Cryptochrome Regulation of Flowering in Arabidopsis. *The Plant Cell*, 20(2), 292 –306.

Ljung, K., 2005. Sites and Regulation of Auxin Biosynthesis in Arabidopsis Roots. *THE PLANT CELL* , 17(4), 1090–1104.

Ljung, Karin et al., 2002. Biosynthesis, conjugation, catabolism and homeostasis of indole-3-acetic acid in Arabidopsis thaliana. *Plant Molecular Biology*, 49(3-4), 249–272.

Lorrain, S. et al., 2008. Phytochrome-mediated inhibition of shade avoidance involves degradation of growth-promoting bHLH transcription factors. *The Plant Journal*, 53(2), 312–323.

de Lucas, M. et al., 2008. A molecular framework for light and gibberellin control of cell elongation. *Nature*, 451(7177), 480–484.

Luccioni, L.G. et al., 2002. Brassinosteroid Mutants Uncover Fine Tuning of Phytochrome Signaling. *Plant Physiology*, 128(1), 173 –181.

Ma, L. et al., 2002. Genomic Evidence for COP1 as a Repressor of Light-Regulated Gene Expression and Development in Arabidopsis. *The Plant Cell*, 14(10), 2383 –2398.

Ma, L. et al., 2001. Light Control of Arabidopsis Development Entails Coordinated Regulation of Genome Expression and Cellular Pathways. *The Plant Cell*, 13(12), 2589 –2607.

Maier, A., 2011. Genetic and Biochemical Characterization of COP1/SPA Function in Arabidopsis Photomorphogenesis. *PhD Thesis*.

Martínez-García, J.F., Huq, E. & Quail, Peter H., 2000. Direct Targeting of Light Signals to a Promoter Element-Bound Transcription Factor. *Science*, 288(5467), 859 –863.

Mashiguchi, K. et al., 2011. The main auxin biosynthesis pathway in Arabidopsis. *Proceedings of the National Academy of Sciences*, 108(45), 18512–18517.

McNellis, T.W., 1994. Genetic and Molecular Analysis of an Allelic Series of cop1 Mutants Suggests Functional Roles for the Multiple Protein Domains. *THE PLANT CELL*, 6(4), 487–500.

Meller, S., 2011. Biochemical and functional analyses of COP1 and SPA proteins in Arabidopsis thaliana. *Master Thesis*. University of Cologne.

Mikkelsen, M. D., 2000. Cytochrome P450 CYP79B2 from Arabidopsis Catalyzes the Conversion of Tryptophan to Indole-3-acetaldoxime, a Precursor of Indole Glucosinolates and Indole-3-acetic Acid. *Journal of Biological Chemistry*, 275(43), 33712–33717.

Mikkelsen, Michael Dalgaard, Naur, P. & Halkier, B.A., 2004. Arabidopsis mutants in the C-S lyase of glucosinolate biosynthesis establish a critical role for indole-3-acetaldoxime in auxin homeostasis. *The Plant Journal*, 37(5), 770–777.

Moon, J. et al., 2008. PIF1 directly and indirectly regulates chlorophyll biosynthesis to optimize the greening process in Arabidopsis. *Proceedings of the National Academy of Sciences*, 105(27), 9433–9438.

Morris, D.A. et al., 1991. Effects of Inhibitors of Protein Synthesis on Transmembrane Auxin Transport in Cucurbita pepo L. Hypocotyl Segments. *Journal of Experimental Botany*, 42(6), 773–783.

Nagy, F. & Schäfer, E., 2002. Phytochrome promote photomorphogenesis by differentially regulated interacting signaling pathways in higher plants. *Annual Review of Plant Biology*, 53(1), 329–355.

Nakagawa, T., Kurose, T., et al., 2007 a. Development of series of gateway binary vectors, pGWBs, for realizing efficient construction of fusion genes for plant transformation. *Journal of Bioscience and Bioengineering*, 104(1), 34–41.

Nakagawa, T., Suzuki, T., et al., 2007 b. Improved Gateway Binary Vectors: High-Performance Vectors for Creation of Fusion Constructs in Transgenic Analysis of Plants. *Bioscience, Biotechnology, and Biochemistry*, 71(8), 2095–2100.

Nelson, D.C. et al., 2000. FKF1, a Clock-Controlled Gene that Regulates the Transition to Flowering in Arabidopsis. *Cell*, 101(3), 331–340.

Nozue, K., Harmer, S.L. & Maloof, J.N., 2011. Genomic Analysis of Circadian Clock-, Light-, and Growth-Correlated Genes Reveals PHYTOCHROME-INTERACTING FACTOR5 as a Modulator of Auxin Signaling in Arabidopsis. *Plant Physiology*, 156(1), 357 –372.

Okushima, Y., 2005. Functional Genomic Analysis of the AUXIN RESPONSE FACTOR Gene Family Members in Arabidopsis thaliana: Unique and Overlapping Functions of ARF7 and ARF19. *THE PLANT CELL*, 17(2), 444–463.

Osterlund, M.T. et al., 2000. Targeted destabilization of HY5 during light-regulated development of Arabidopsis. *Nature*, 405(6785), 462–466.

Overvoorde, P.J., 2005. Functional Genomic Analysis of the AUXIN/INDOLE-3-ACETIC ACID Gene Family Members in Arabidopsis thaliana. *THE PLANT CELL*, 17(12), 3282–3300.

Oyama, T., Shimura, Y. & Okada, K., 1997. The Arabidopsis HY5 gene encodes a bZIP protein that regulates stimulus-induced development of root and hypocotyl. *Genes & Development*, 11(22), 2983 – 2995.

Park, J.-E. et al., 2007. An Arabidopsis GH3 Gene, Encoding an Auxin-Conjugating Enzyme, Mediates Phytochrome B-Regulated Light Signals in Hypocotyl Growth. *Plant and Cell Physiology*, 48(8), 1236–1241.

Parks, B.M., Hoecker, U. & Spalding, E.P., 2001b. Light-Induced Growth Promotion by SPA1 Counteracts Phytochrome-Mediated Growth Inhibition during De-Etiolation. *Plant Physiology*, 126(3), 1291 –1298.

Perrot-Rechenmann, C., 2010. Cellular Responses to Auxin: Division versus Expansion. *Cold Spring Harbor Perspectives in Biology*, 2(5).

Pfeiffer, A. et al., 2012. Interaction with plant transcription factors can mediate nuclear import of phytochrome B. *Proceedings of the National Academy of Sciences*, 109(15), 5892–5897.

Pierik, R. et al., 2009. Auxin and Ethylene Regulate Elongation Responses to Neighbor Proximity Signals Independent of Gibberellin and DELLA Proteins in Arabidopsis. *PLANT PHYSIOLOGY*, 149(4), 1701–1712.

Quail, P H et al., 1995. Phytochromes: photosensory perception and signal transduction. *Science (New York, N.Y.)*, 268(5211), 675–680.

Rahman, A. et al., 2007. Auxin, actin and growth of the Arabidopsis thaliana primary root. *The Plant Journal*, 50(3), 514–528.

Ranjan, A. et al., 2011. The Arabidopsis repressor of light signaling SPA1 acts in the phloem to regulate seedling de-etiolation, leaf expansion and flowering time. *Development*, 138(9), 1851–1862.

Reed, J. W. et al., 1996. Phytochrome B Affects Responsiveness to Gibberellins in Arabidopsis. *Plant Physiology*, 112(1), 337 –342.

Reed, Jason W et al., 1993. Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout Arabidopsis development. *THE PLANT CELL*, 5(2), 147–157.

Remington, D.L., 2004. Contrasting Modes of Diversification in the Aux/IAA and ARF Gene Families. *PLANT PHYSIOLOGY*, 135(3), 1738–1752.

Rizzini, L. et al., 2011. Perception of UV-B by the Arabidopsis UVR8 Protein. *Science*, 332(6025), 103–106.

Roig-Villanova, I. et al., 2006. Identification of Primary Target Genes of Phytochrome Signaling. Early Transcriptional Control during Shade Avoidance Responses in Arabidopsis. *PLANT PHYSIOLOGY*, 141(1), 85–96.

Roig-Villanova, I. et al., 2007. Interaction of shade avoidance and auxin responses: a role for two novel atypical bHLH proteins. *EMBO J*, 26(22), 4756–4767.

Rösler, J., Klein, I. & Zeidler, M., 2007. Arabidopsis fhl/fhy1 double mutant reveals a distinct cytoplasmic action of phytochrome A. *Proceedings of the National Academy of Sciences*, 104(25), 10737 –10742.

Ruckle, M.E., DeMarco, S.M. & Larkin, R.M., 2007. Plastid Signals Remodel Light Signaling Networks and Are Essential for Efficient Chloroplast Biogenesis in Arabidopsis. *The Plant Cell*, 19(12), 3944 –3960.

Sahm, J., 2010. Schattenvermeidungsantwort bei Pflanzen: Interaktion von Licht- und Auxin-Signaltransduktion bei der Pflanze Arabidopsis thaliana. *Examensarbeit*. Universität zu Köln, 2010

Saijo, Y. et al., 2003. The COP1–SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. *Genes & Development*, 17(21), 2642 –2647.

Saito, K., Watahiki, M.K. & Yamamoto, K.T., 2007. Differential expression of the auxin primary response gene MASSUGU2/IAA19during tropic responses of Arabidopsis hypocotyls. *Physiologia Plantarum*, 130(1), 148–156.

Salisbury, F.J. et al., 2007. Phytochrome coordinates Arabidopsis shoot and root development. *The Plant Journal*, 50(3), 429–438.

Salter, M.G., Franklin, Keara A. & Whitelam, Garry C., 2003. Gating of the rapid shade-avoidance response by the circadian clock in plants. *Nature*, 426(6967), 680–683.

Sato, A. & Yamamoto, K.T., 2008. Overexpression of the non-canonical Aux/IAA genes causes auxin-related aberrant phenotypes in Arabidopsis. *Physiologia Plantarum*, 133(2), 397–405.

Savaldi-Goldstein, S., Peto, C. & Chory, J., 2007. The epidermis both drives and restricts plant shoot growth. *Nature*, 446(7132), 199–202.

Schenck, D. et al., 2010. Rapid Auxin-Induced Cell Expansion and Gene Expression: A Four-Decade-Old Question Revisited. *Plant Physiology*, 152(3), 1183 –1185.

Sellaro, R., Yanovsky, Marcelo J. & Casal, Jorge J., 2011. Repression of shade-avoidance reactions by sunfleck induction of HY5 expression in Arabidopsis. *The Plant Journal*, 68(5), 919–928.

Seo, H.S. et al., 2003. LAF1 ubiquitination by COP1 controls photomorphogenesis and is stimulated by SPA1. *Nature*, 423(6943), 995–999.

Seo, H.S. et al., 2004. Photoreceptor ubiquitination by COP1 E3 ligase desensitizes phytochrome A signaling. *Genes & Development*, 18(6), 617 –622.

Sessa, G. et al., 2005. A dynamic balance between gene activation and repression regulates the shade avoidance response in Arabidopsis. *Genes & Development*, 19(23), 2811 –2815.

Sharrock, R. A., 2002. Patterns of Expression and Normalized Levels of the Five Arabidopsis Phytochromes. *PLANT PHYSIOLOGY*, 130(1), 442–456.

Sharrock, R. A. & Clack, T., 2004. Heterodimerization of type II phytochromes in Arabidopsis. *Proceedings of the National Academy of Sciences*, 101(31), 11500–11505.

Shin, J. et al., 2009. Phytochromes promote seedling light responses by inhibiting four negatively-acting phytochrome-interacting factors. *Proceedings of the National Academy of Sciences*, 106(18), 7660 –7665.

Sibout, R. et al., 2006. Opposite Root Growth Phenotypes of hy5 versus hy5 hyh Mutants Correlate with Increased Constitutive Auxin Signaling. *PLoS Genetics*, 2(11), S.e202.

Smith, H., Xu, Y & Quail, P H, 1997. Antagonistic but complementary actions of phytochromes A and B allow seedling de-etiolation. *PLANT PHYSIOLOGY*, 114(2), 637–641.

Smolen, G. & Bender, J., 2002. Arabidopsis Cytochrome P450 cyp83B1 Mutations Activate the Tryptophan Biosynthetic Pathway. *Genetics*, 160(1), 323 –332.

Soh, M.S. et al., 1999. Regulation of both light- and auxin-mediated development by the Arabidopsis IAA3/SHY2 gene. *Journal of Plant Biology*, 42(3), 239–246.

Somers, D. E., 2004. The F-Box Protein ZEITLUPE Confers Dosage-Dependent Control on the Circadian Clock, Photomorphogenesis, and Flowering Time. *THE PLANT CELL*, 16(3), 769–782.

Somers, David E et al., 2000. ZEITLUPE Encodes a Novel Clock-Associated PAS Protein from Arabidopsis. *Cell*, 101(3), 319–329.

Sorin, C. et al., 2009. ATHB4, a regulator of shade avoidance, modulates hormone response in Arabidopsis seedlings. *The Plant Journal*, 59(2), 266–277.

Spartz, A.K. et al., 2012. The SAUR19 subfamily of SMALL AUXIN UP RNA genes promote cell expansion. *The Plant Journal*, advanced online.

Staswick, P.E., 2005. Characterization of an Arabidopsis Enzyme Family That Conjugates Amino Acids to Indole-3-Acetic Acid. *THE PLANT CELL*, 17(2), 616–627.

Staswick, P.E., Tiryaki, I. & Rowe, M.L., 2002. Jasmonate Response Locus JAR1 and Several Related Arabidopsis Genes Encode Enzymes of the Firefly Luciferase Superfamily That Show Activity on Jasmonic, Salicylic, and Indole-3-Acetic Acids in an Assay for Adenylation. *THE PLANT CELL*, 14(6), 1405–1415.

Steindler, C. et al., 1999. Shade avoidance responses are mediated by the ATHB-2 HD-zip protein, a negative regulator of gene expression. *Development*, 126(19), 4235 –4245.

Stepanova, A. N. et al., 2011. The Arabidopsis YUCCA1 Flavin Monooxygenase Functions in the Indole-3-Pyruvic Acid Branch of Auxin Biosynthesis. *THE PLANT CELL* , 23(11), 3961–3973.

Stepanova, Anna N. et al., 2008. TAA1-Mediated Auxin Biosynthesis Is Essential for Hormone Crosstalk and Plant Development. *Cell*, 133(1), 177–191.

Strasser, B., Sanchez-Lamas, M., et al., 2010. Arabidopsis thaliana life without phytochromes. *Proceedings of the National Academy of Sciences*, 107(10), 4776–4781.

Swarup, R. et al., 2004. Structure-Function Analysis of the Presumptive Arabidopsis Auxin Permease AUX1. *The Plant Cell* , 16(11), 3069 –3083.

Tam, Y.Y., Epstein, E. & Normanly, J., 2000. Characterization of Auxin Conjugates in Arabidopsis. Low Steady-State Levels of Indole-3-Acetyl-Aspartate, Indole-3-Acetyl-Glutamate, and Indole-3-Acetyl-Glucose. *PLANT PHYSIOLOGY*, 123(2), 589–596.

Tan, X. et al., 2007. Mechanism of auxin perception by the TIR1 ubiquitin ligase. *Nature*, 446(7136), 640–645.

Tao, Y. et al., 2008. Rapid Synthesis of Auxin via a New Tryptophan-Dependent Pathway Is Required for Shade Avoidance in Plants. *Cell*, 133(1), 164–176.

Tatematsu, K. et al., 2004. MASSUGU2 Encodes Aux/IAA19, an Auxin-Regulated Protein That Functions Together with the Transcriptional Activator NPH4/ARF7 to Regulate Differential Growth Responses of Hypocotyl and Formation of Lateral Roots in Arabidopsis thaliana. *The Plant Cell* , 16(2), 379 –393.

Tepperman, J.M. et al., 2001. Multiple transcription-factor genes are early targets of phytochrome A signaling. *Proceedings of the National Academy of Sciences*, 98(16), 9437 –9442.

Tepperman, J.M., Hwang, Y.-S. & Quail, Peter H., 2006. phyA dominates in transduction of red-light signals to rapidly responding genes at the initiation of Arabidopsis seedling de-etiolation. *The Plant Journal*, 48(5), 728–742.

Tian, Q. & Reed, Jason W, 1999. Control of auxin-regulated root development by the Arabidopsis thaliana SHY2/IAA3 gene. *Development*, 126, 711–721.

Ulmasov, T, Murfett, J., et al., 1997a. Aux/IAA Proteins Repress Expression of Reporter Genes Containing Natural and Highly Active Synthetic Auxin Response Elements. *THE PLANT CELL*, 9(11), 1963–1971.

Ulmasov, Tim, Hagen, G. & Guilfoyle, T.J., 1997b. ARF1, a Transcription Factor That Binds to Auxin Response Elements. *Science*, 276(5320), 1865 –1868.

Valverde, F. et al., 2004. Photoreceptor Regulation of CONSTANS Protein in Photoperiodic Flowering. *Science*, 303(5660), 1003 –1006.

Vandenbussche, F. et al., 2005. Reaching out of the shade. *Current Opinion in Plant Biology*, 8(5), 462–468.

Walcher, C.L. & Nemhauser, J.L., 2012. Bipartite Promoter Element Required for Auxin Response. *Plant Physiology*, 158(1), 273 –282.

Wang, H. et al., 2001. Direct Interaction of Arabidopsis Cryptochromes with COP1 in Light Control Development. *Science*, 294(5540), 154 –158.

Wang, J.-G. et al., 2011. FAR-RED INSENSITIVE219 Modulates CONSTITUTIVE PHOTOMORPHOGENIC1 Activity via Physical Interaction to Regulate Hypocotyl Elongation in Arabidopsis. *PLANT PHYSIOLOGY*, 156(2), 631–646.

Wei, N. & Deng, X.W., 1996. The Role of the COP/DET/FUS Genes in Light Control of Arabidopsis Seedling Development. *Plant Physiology*, 112(3), 871 –878.

Wenkel, S. et al., 2006. CONSTANS and the CCAAT Box Binding Complex Share a Functionally Important Domain and Interact to Regulate Flowering of Arabidopsis. *The Plant Cell*, 18(11), 2971 – 2984.

Wollenberg, A.C. et al., 2008. Acceleration of Flowering during Shade Avoidance in Arabidopsis Alters the Balance between FLOWERING LOCUS C-Mediated Repression and Photoperiodic Induction of Flowering. *Plant Physiology*, 148(3), 1681 –1694.

Won, C. et al., 2011. Conversion of tryptophan to indole-3-acetic acid by TRYPTOPHAN AMINOTRANSFERASES OF ARABIDOPSIS and YUCCAs in Arabidopsis. *Proceedings of the National Academy of Sciences*, 108(45), 18518–18523.

Woodward, A.W. & Bartel, B., 2005. Auxin: Regulation, Action, and Interaction. *Annals of Botany*, 95(5), 707–735.

Wu, G. et al., 2010. A role for ABCB19-mediated polar auxin transport in seedling photomorphogenesis mediated by cryptochrome 1 and phytochrome B. *The Plant Journal*, 62(2), 179–191.

Xu, T. et al., 2010. Cell Surface- and Rho GTPase-Based Auxin Signaling Controls Cellular Interdigitation in Arabidopsis. *Cell*, 143(1), 99–110.

Yamada, M. et al., 2009. The TRANSPORT INHIBITOR RESPONSE2 Gene Is Required for Auxin Synthesis and Diverse Aspects of Plant Development. *Plant Physiology*, 151(1), 168 –179.

Yamaguchi, A. et al., 2005. TWIN SISTER OF FT (TSF) Acts As a Floral Pathway Integrator Redundantly with FT. *Plant and Cell Physiology*. doi: 10.1093/pcp/pci151

Yang, H.-Q., Tang, R.-H. & Cashmore, A.R., 2001. The Signaling Mechanism of Arabidopsis CRY1 Involves Direct Interaction with COP1. *The Plant Cell* , 13(12), 2573 –2587.

Yang, J., Lin, R., Sullivan, J., et al., 2005. Light Regulates COP1-Mediated Degradation of HFR1, a Transcription Factor Essential for Light Signaling in Arabidopsis. *The Plant Cell*, 17(3), 804 –821.

Yang, J., Lin, R., Hoecker, U., et al., 2005a. Repression of light signaling by Arabidopsis SPA1 involves post-translational regulation of HFR1 protein accumulation. *The Plant Journal*, 43(1), 131–141.

Yang, K.-Y. et al., 2003. Overexpression of a Mutant Basic Helix-Loop-Helix Protein HFR1, HFR1-ΔN105, Activates a Branch Pathway of Light Signaling in Arabidopsis. *Plant Physiology*, 133(4), 1630 –1642.

Yang, X. et al., 2004. The IAA1 protein is encoded by AXR5 and is a substrate of SCFTIR1. *The Plant Journal*, 40(5), 772–782.

Yang, Y. et al., 2008. Inactive Methyl Indole-3-Acetic Acid Ester Can Be Hydrolyzed and Activated by Several Esterases Belonging to the AtMES Esterase Family of Arabidopsis. *PLANT PHYSIOLOGY*, 147(3), 1034–1045.

Yanovsky, M. J., Casal, J. J. & Whitelam, G. C., 1995. Phytochrome A, phytochrome B and HY4 are involved in hypocotyl growth responses to natural radiation in Arabidopsis: weak de-etiolation of the phyA mutant under dense canopies. *Plant, Cell and Environment*, 18(7), 788–794.

Yanovsky, Marcelo J. & Kay, S.A., 2002. Molecular basis of seasonal time measurement in Arabidopsis. *Nature*, 419(6904), 308–312.

Zhang, J., Nodzynski, T., et al., 2009. PIN phosphorylation is sufficient to mediate PIN polarity and direct auxin transport. *Proceedings of the National Academy of Sciences*, 107(2), 918–922.

Zhang, L.-Y., Bai, M.-Y., et al., 2009. Antagonistic HLH/bHLH Transcription Factors Mediate Brassinosteroid Regulation of Cell Elongation and Plant Development in Rice and Arabidopsis. *The Plant Cell*, 21(12), 3767 –3780.

Zhao, Y., 2001. A Role for Flavin Monooxygenase-Like Enzymes in Auxin Biosynthesis. *Science*, 291(5502), 306–309.

Zhao, Y., 2011. Auxin Biosynthesis: A Simple Two-Step Pathway Converts Tryptophan to Indole-3-Acetic Acid in Plants. *Molecular Plant*, 5(2), 334–338.

Zhu, D. et al., 2008. Biochemical Characterization of Arabidopsis Complexes Containing CONSTITUTIVELY PHOTOMORPHOGENIC1 and SUPPRESSOR OF PHYA Proteins in Light Control of Plant Development. *THE PLANT CELL*, 20(9), 2307–2323.

VI. Supplement

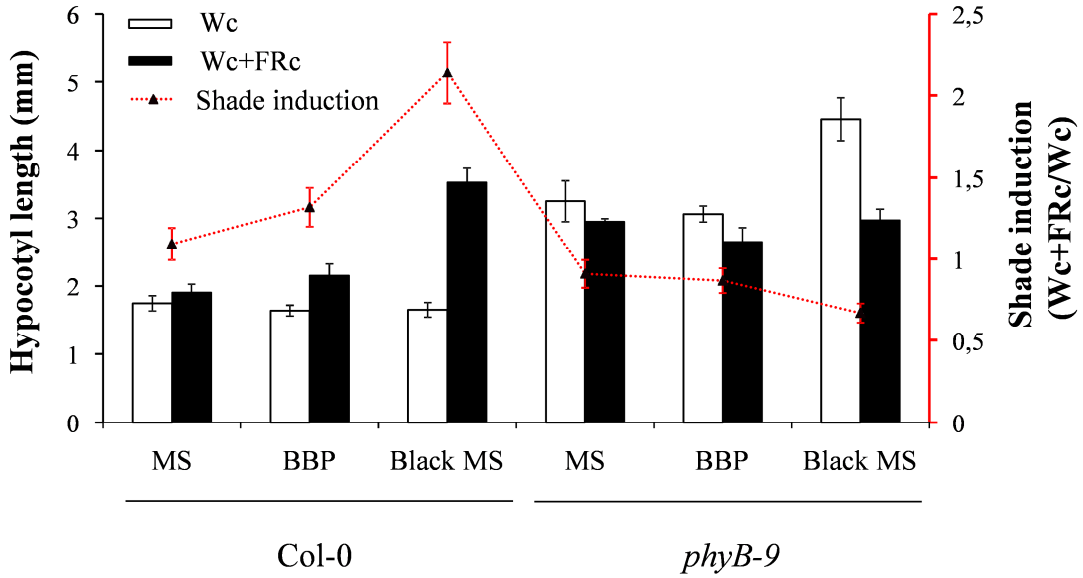

Figure S1: Hypocotyl elongation response of seedlings in low R:FR conditions is enhanced on black MS plates. WT and *phyB-9* seedlings were grown on MS plates, on MS plates with blackened bottom (BBP) or on black MS plates. Seedlings were treated for three days with Wc followed by additional three days of low R:FR treatment or Wc. Hypocotyls were measured, the mean was calculated and expressed ± SEM (n > 15).

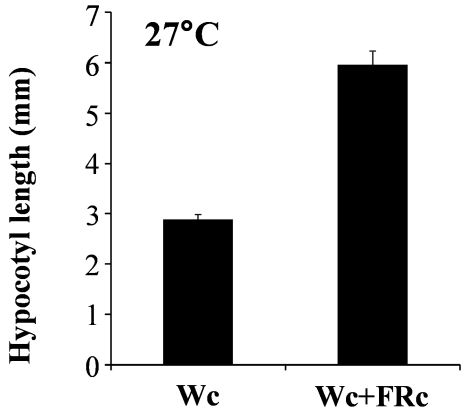

Figure S2: Hypocotyls of WT seedlings responded to low R:FR treatment at 27°C. WT seedlings were grown at elevated temperatures (27°C) for 3 days in Wc and shifted to low R:FR or kept in Wc for additinal 3 days. These conditions were applied for the DR5::GUS experiment to boost basal levels of auxin in the plants.

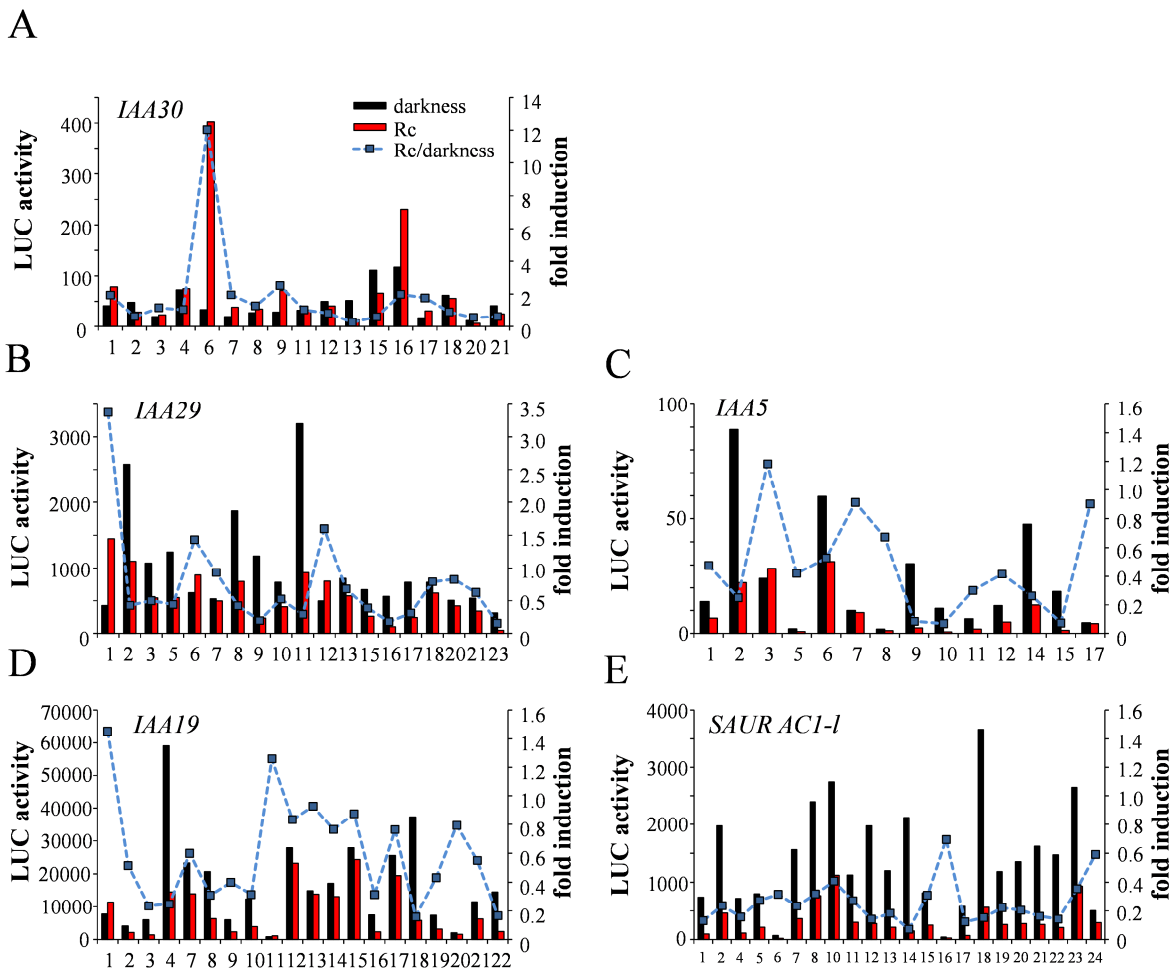

Figure S3: Activity of auxin-responsive promoters in darkness and light. The individual results obtained from quantitative luciferase analyses (qLUC) for each T2 line in the set of promoters from auxin-induced genes. Shown is the LUC activity in darkness (black bars), after 24 hours of Rc treatment (red bars) and the fold-induction (Rc/darkness; in blue).

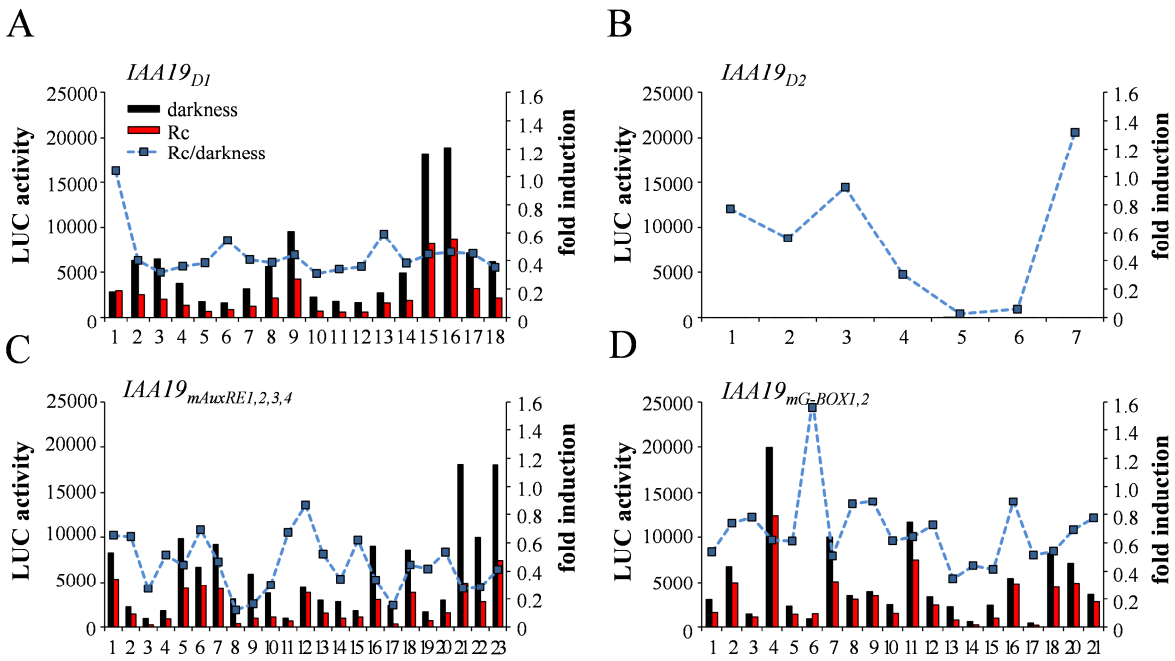

Figure S4: Activity of *IAA19* promoter constructs in darkness and light. The individual results obtained from quantitative luciferase analyses (qLUC) for each T2 line from the set of *IAA19* deletions and mutation constructs. Shown is the LUC activity in darkness (black bars), after 24 hours of Rc treatment (red bars) and the fold-induction (Rc/darkness; in blue).

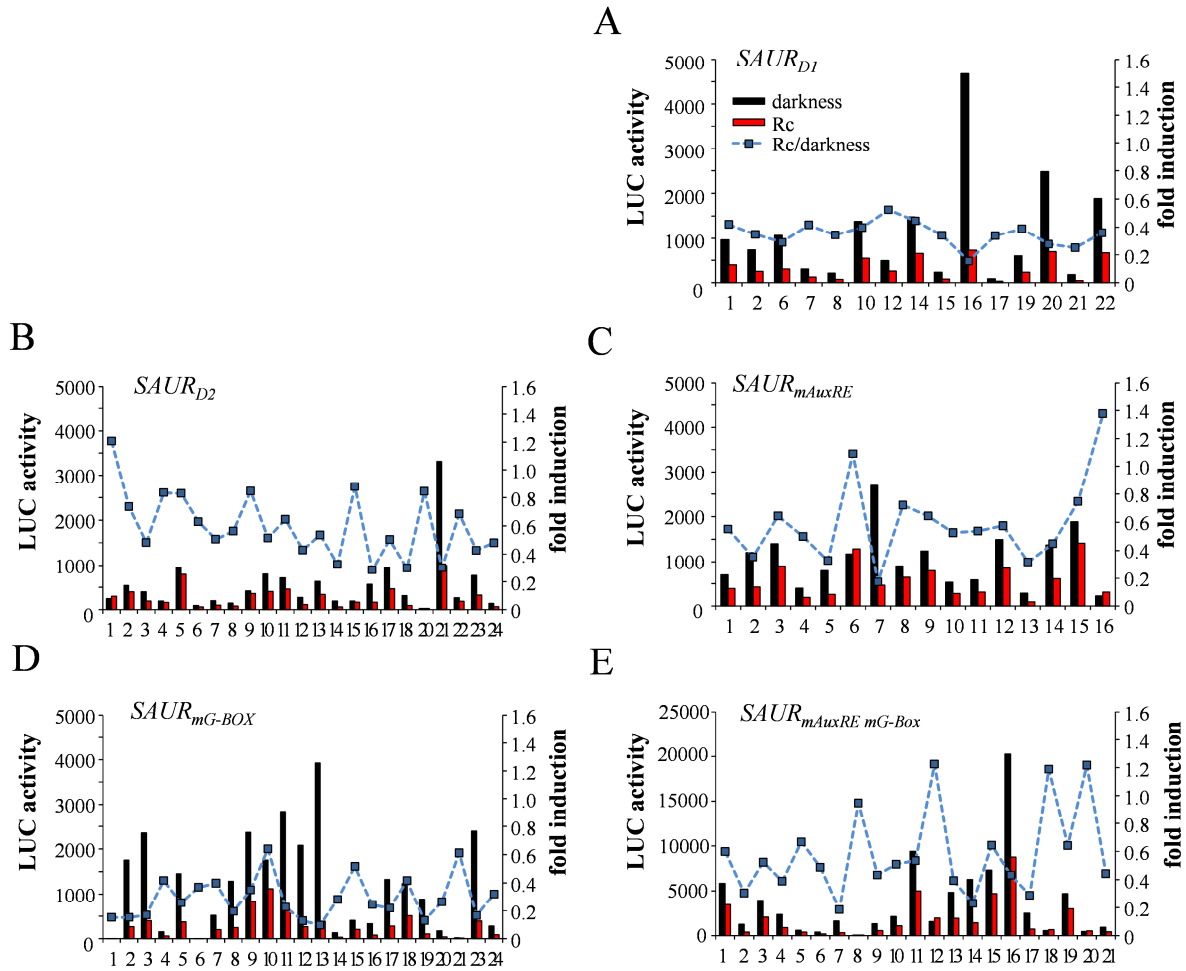

Figure S5: Activity of *SAUR-AC1-l* promoter constructs in darkness and light. The individual results obtained from quantitative luciferase analyses (qLUC) for each T2 line from the set of *SAUR-AC1-l* deletions and mutation constructs. Shown is the LUC activity in darkness (black bars), after 24 hours of Rc treatment (red bars) and the fold-induction (Rc/darkness; in blue).

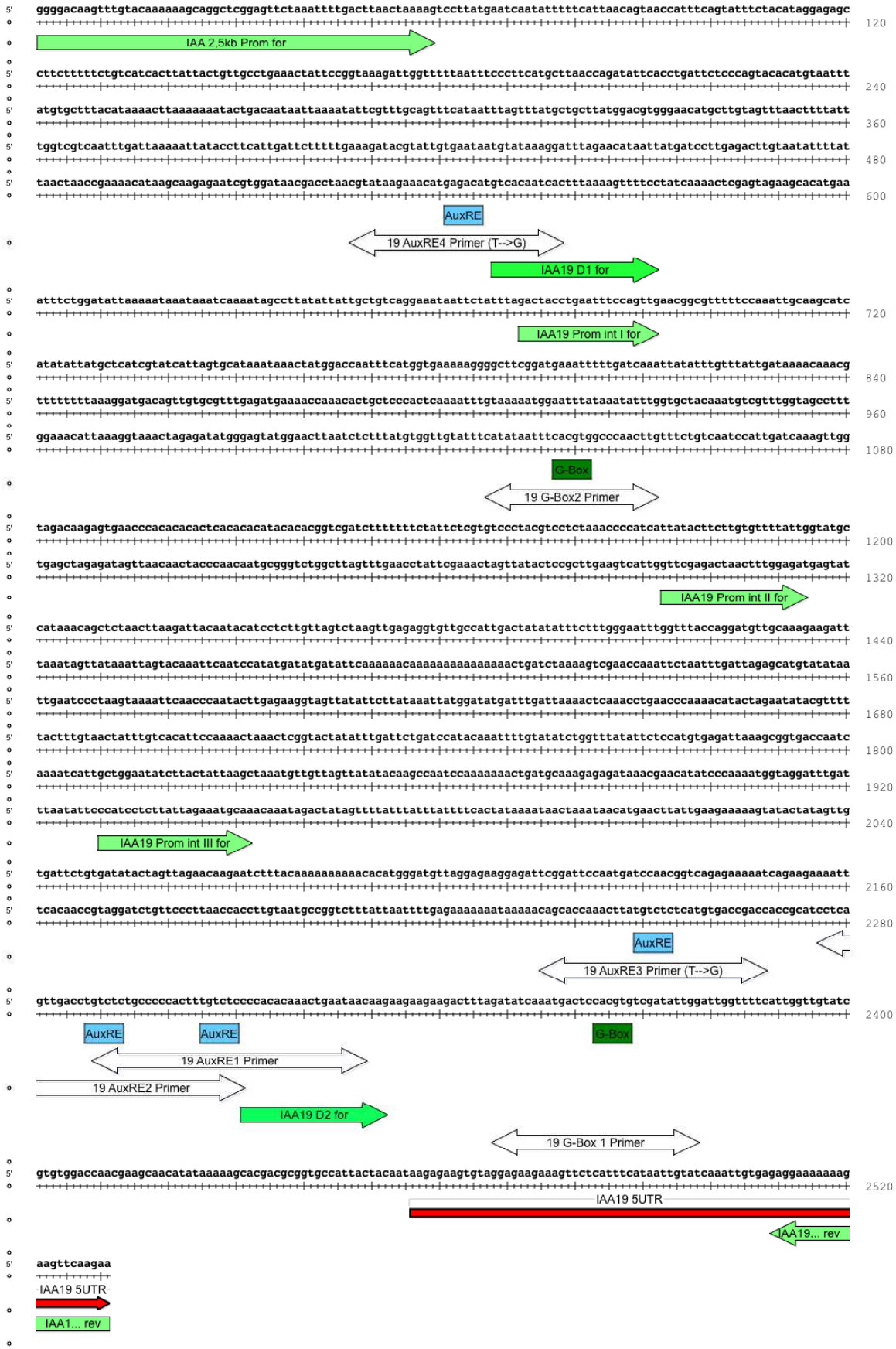

Figure S6: Representation of the *IAA19* 2.5kb promoter fragment. The 5' stand is shown, primers are depicted in light green (for amplification and sequencing) and white (for point-mutation). AuxREs are highlighted in blue, G-Boxes in dark green.

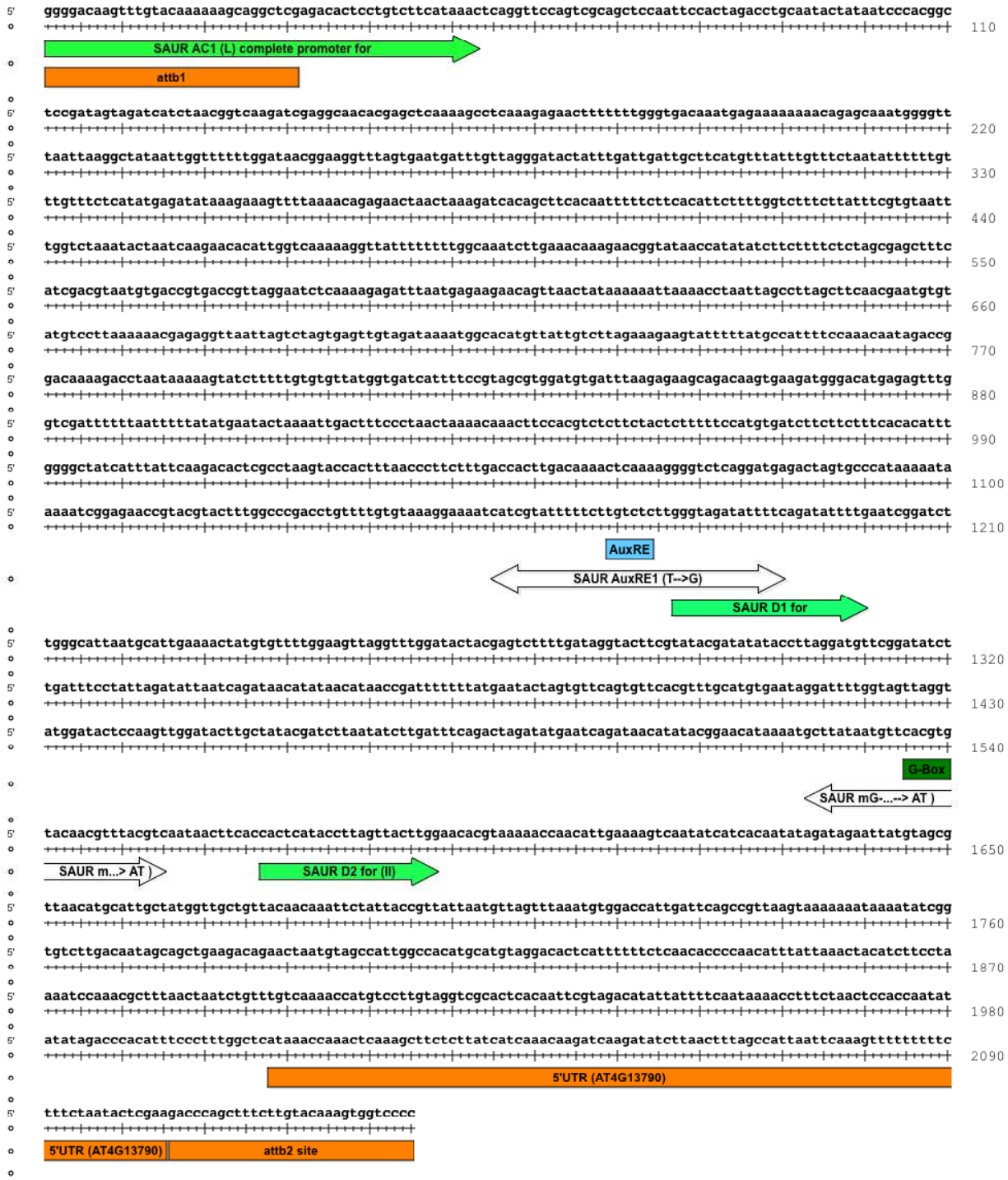

Figure S7: Representation of the *SAUR* promoter fragment. The 5' stand is shown, primers are depicted in light green (for amplification and sequencing) and white (for point-mutation). AuxREs are highlighted in blue, G-Boxes in dark green. The attb1 and b2 sites are highlighted in orange.

VII. Danksagung

"*Bernard of Chartres used to say that we are like dwarfs on the shoulders of giants, so that we can see more than they, and things at a greater distance, not by virtue of any sharpness of sight on our part, or any physical distinction, but because we are carried high and raised up by their giant size.*" ***Metalogicon****, John of Salisbury, 1159*

Above all, I would like to thank Prof. Dr. Ute Höcker for the opportunity to complete my thesis in her lab and her great scientific guidance. I especially appreciate the way that Ute supported me with my ideas about my future working life. Thanks to Prof. Dr. Martin Hülskamp for being second examiner of my thesis and Prof. Dr. Wolfgang Werr for being head of my examination committee.

Thanks to all of my great colleagues from the AG Höcker for the exceptional working atmosphere, the help that I received and all the fun! I especially want to mention the outstanding *mensa project* and the *Thirsty Thursday get-togethers*. My special thanks to Petra for having always been ready to help and for the reliable release of her funny daily editorial. Alex could have hardly been more warm-hearted and supportive as a colleague and friend. Thanks to Lennart who shared an office with me for some time until water eventually flooded the setup. I also want to thank Aashish for fruitful discussions and Kirsten for the support when it all began. Thanks to Martin for the scientific discussions and a lot of funny dialogues. I also want to thank Stefan for his way to keep reflecting things and for commenting on the script. Also thanks to Leonie for comments on the script, for keeping me company, especially in "Maria in der Aue" and above all for always organising the refreshing drinks. I would like to thank Xu for the nice chats, both scientific and non-scientific, not to mention the self-cooked food she kept sharing with me. I also want to thank Song for his friendliness and lots of help. Thanks to Melina for her lively contribution to office 1 and all gossips about Krefeld and the rest of the world. I also want to thank Gabi for her motherly care and strictness. Thanks to Laura, her dry sense of humor and for always being very nice. Many thanks to the two genial and natural guys I was fortunate enough to guide through their time as candidates, Jan and Stephen. Thanks especially for your dedication to the shade project that saved the day.

I also want to express my indebtedness to the professional greenhouse staff of the University and the AG Höcker Hiwis, thanks a lot for the plant work and everything else that you did for me! Thanks also to Alex from the AG Maurino, who went back and forth with me uncountable times with the RE5, keeping me delightful company. Many thanks to all the other guys in the botanical institute: for the friendly and enjoyable interactions.

My family has always been an incredible support all my life and I cannot be clear enough about how much they all mean to me. Thanks to my siblings, their beloved once and especially my father for all the help they provided the last thirty years.

Sara. Thanks for being the person you are. Without you, all pictures would just be colourless frames.

This thesis is dedicated to my mother, Ruth, who passed away before her time and who I sorely miss.

Erklärung

„Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit - einschließlich Tabellen, Karten und Abbildungen -, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie – abgesehen von unten angegebenen Teilpublikationen – noch nicht veröffentlicht worden ist sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen der Promotionsordnung sind mir bekannt.
Die von mir vorgelegte Dissertation ist von Prof. Dr. Ute Höcker betreut worden.“

Sebastian Rolauffs