Influence of light and cytokinin on organellar phage-type RNA polymerase transcript levels and transcription of organellar genes in *Arabidopsis thaliana*

DISSERTATION

zur Erlangung des akademischen Grades

d o c t o r r e r u m n a t u r a l i u m

(Dr. rer. nat.)

im Fach Biologie

eingereicht an der

Mathematisch-Naturwissenschaftlichen Fakultät I

der Humboldt-Universität zu Berlin

von

Diplom-Ingenieurin Biotechnologie Liliana Borsellino

Präsident der Humboldt-Universität zu Berlin
Prof. Dr. Jan-Hendrik Olbertz

Dekan der Mathematisch-Naturwissenschaftlichen Fakultät I
Prof. Dr. Andreas Herrmann

Gutachter: 1. Prof. Dr. Thomas Börner

2. PD Dr. Thomas Pfannschmidt

3. Prof. Dr. Wolfgang Schuster

Tag der mündlichen Prüfung: 13.12.2011

"Discovery consists of seeing what everybody has seen and thinking what nobody has thought."

Albert Szent-Gyorgyi (1962)

Abstract

Light and plant hormones such as cytokinins are essential for plant growth and development. Only little information is available about how these signals influence the transcription of organellar genes. *Arabidopsis thaliana* possesses three nuclear-encoded phage-type RNA polymerases (RpoT) for organellar transcription. They are imported into plastids (RpoTp), mitochondria (RpoTm), or into both organelles (RpoTmp). Besides the two nuclear-encoded plastid polymerases (NEP), plastids contain an additional plastid-encoded RNA polymerase (PEP), which needs additional sigma factors for promoter recognition.

Interested in the expression of *RpoT* genes and NEP-transcribed plastid genes in response to light we analyzed transcript levels of *RpoT* and *rpoB* genes in 7-day-old wild-type plants under different light conditions by quantitative real-time-PCR. The observed changes in transcript accumulation indicated that red, blue, and green light differentially stimulated the expression of all three *RpoT* genes. Further analyses using different photoreceptor mutants showed that light induction of *RpoT* gene expression is surprisingly complex based on a network of multiple photoreceptors and downstream pathways.

Cytokinin signals are perceived by the histidine kinase (AHK) receptor family. There exist three different membrane-bound receptors: AHK2, AHK3 and AHK4/CRE1. These receptors are part of a two-component signaling system which transfers signals via phosphorelay mechanisms. Interested in the potential role of AHK2, AHK3 and AHK4/CRE1 in the transduction of cytokinin signals into the chloroplast, we analyzed the influence of cytokinin on plastidial transcription in receptor mutants. To gain more information on how plastid transcription by PEP is regulated by cytokinin, the influence of cytokinin in sigma factor mutants was also studied.

Keywords:

phage-type RNA polymerases

organellar gene transcription

photoreceptors

light-induction

cytokinin

Zusammenfassung

Licht und Pflanzenhormone wie Cytokinine sind essentiell für das Wachstum und die Entwicklung von Pflanzen. Es ist nur wenig darüber bekannt, wie sie die Transkription organellärer Gene beeinflussen. In *Arabidopsis thaliana* gibt es drei kernkodierte Phagentyp-RNA-Polymerasen (RpoT), welche für die organelläre Transkription verantwortlich sind. Diese werden in die Plastiden (RpoTp), die Mitochondrien (RpoTm) oder zu beiden Organellen (RpoTmp) transportiert. Neben den beiden kernkodierten RNA-Polymerasen (NEP) existiert in den Plastiden eine plastidärkodierte RNA-Polymerase (PEP), welche zusätzliche Sigmafaktoren zur Promotererkennung benötigt.

Um die Lichtabhängigkeit der Expression der *RpoT* Gene sowie NEP-transkribierter Chloroplastengene zu analysieren, wurde die Akkumulation von *RpoT*- und *rpoB*-Transkripten in 7-Tage alten Keimlingen unter verschiedenen Lichtbedingungen mittels quantitativer real-time PCR untersucht. Die beobachteten Änderungen in der Transkriptakkumulation deuten darauf hin, dass rote, blaue und grüne Wellenlängen die Expression der drei *RpoT* Gene unterschiedlich stark stimulieren. Untersuchungen an verschiedenen Lichtrezeptor-Mutanten zeigten, dass die Lichtinduktion der *RpoT* Genexpression überaus komplex ist und ein interagierendes Netzwerk aus multiplen Photorezeptoren und Transkriptionsfaktoren an der Signalweiterleitung beteiligt ist.

Das Phytohormon Cytokinin wird durch Histidin Kinase Rezeptoren (AHK) detektiert. Es gibt drei unterschiedliche membran-gebundene Rezeptoren: AHK2, AHK3 und AHK4/CRE1. Diese sind Teil eines Zwei-Komponenten-Signalsystems, welches Signale mit Hilfe einer Phosphorylierungskette überträgt. Der Einfluss von Cytokinin auf die plastidäre Transkription wurde mit Hilfe von Cytokininrezeptor-Mutanten untersucht, um die Funktion von AHK2, AHK3 und AHK4/CRE1 zu analysieren. Um weitere Informationen darüber zu erhalten, wie die plastidäre Transkription durch PEP mittels Cytokinin reguliert wird, wurden die Effekte von Cytokinin auf die plastidäre Transkription in Sigmafaktor-Mutanten geprüft.

Schlagwörter:

Phagentyp-RNA-Polymerasen

Organelläre Gentranskription

Photorezeptoren

Lichtinduktion

Cytokinin

Table of content

1 Introduction

Plants and green algae contain plastids, which are organelles that originate from an ancient cyanobacterial endosymbiont (Gray, 1999; Martin *et al.*, 2001). Plastids possess their own genome (plastome) encoding genes important for their function and biogenesis. However, most plastid genes have been transferred to the nucleus during endosymbiontic evolution (Martin *et al.*, 2002; Stegemann *et al.*, 2003; Timmis *et al.*, 2004). Still, plastids have their own transcription machinery (see 1.1; Liere and Börner, 2007a,b; Liere *et al.*, 2011).

The expression of plastid genes is not only regulated post-transcriptionally but also to some extent on the transcriptional level in response to several external and internal stimuli (see reviews by Liere *et al.*, 2011; Barkan, 2011). Nevertheless, how these specific signals are transmitted into the organelles and how they act on the transcription of plastid genes is largely unknown. In this thesis, new data will be presented that shed some light on the mechanisms that regulate organellar gene transcription in higher plants.

1.1 The transcription machinery of plastids

The complex transcription in plastids of dicots is based on two different kinds of RNA polymerases (Figure 1): a nuclear-encoded, phage-type and a plastid-encoded, eubacterial-like RNA polymerase (Maliga, 1998; Hess and Börner, 1999; Shiina *et al.*, 2005; Toyoshima *et al.*, 2005). *Arabidopsis thaliana* possesses three different nuclear-encoded phage-type RNA polymerases. They are encoded by the small family of *RpoT* genes. The gene products are imported into plastids (RpoTp), mitochondria (RpoTm), and are dual-targeted (RpoTmp) (Hedtke *et al.*, 1997, 2000, 2002; Cahoon and Stern, 2001; Kobayashi *et al.*, 2001; Richter *et al.*, 2002). It was shown that RpoTp and RpoTmp represent the nuclear-encoded plastid RNA polymerase (NEP) in dicots (Chang *et al.*, 1999; Kusumi *et al.*, 2004; Azevedo *et al.*, 2008).

The plastid-encoded plastid RNA polymerase (PEP) is a multi-subunit enzyme homologous to bacterial RNA polymerases (Hess and Börner, 1999; Shiina *et al.*, 2005; Liere and Börner, 2007 a,b). Functional PEP complexes consist of five core subunits (2 x α, β, β´, β´´), which are encoded by the *rpoA*, *rpoB*, *rpoC1* and *rpoC2* genes (Serino and Maliga, 1998; Suzuki *et al.*, 2004), which are associated with one of the nuclear-encoded sigma factors for promoter recognition *in vivo*. Six different sigma factors, AtSig1 to AtSig6, are present in *Arabidopsis*, which have partly overlapping functions controlling the transcription of plastid genes (see reviews by Allison, 2000; Lysenko, 2007; Schweer, 2010).

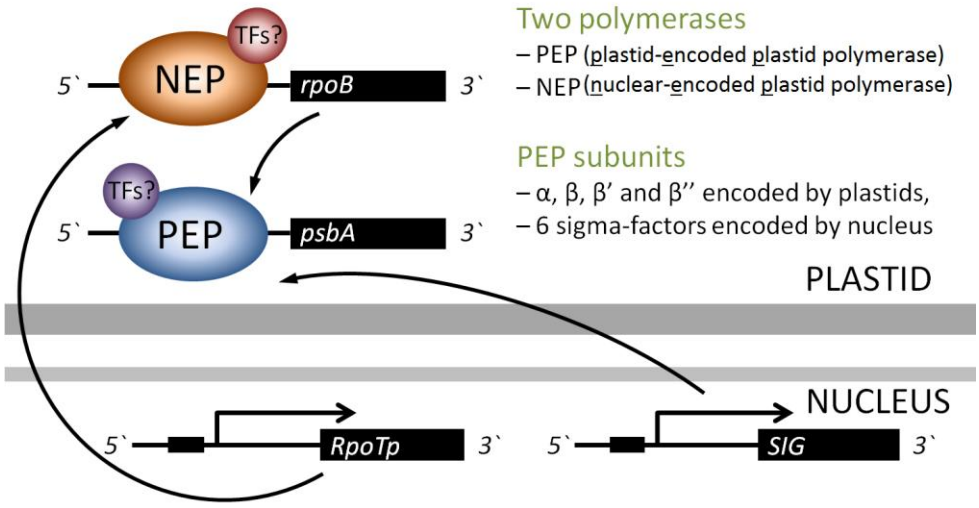

Figure 1: The transcriptional machinery of plastids.

The transcription machinery of plastids consists of two different RNA polymerases. The nuclear-encoded plastid RNA polymerase (NEP), which is related to phage-type single-subunit enzymes and the plastid-encoded plastid RNA polymerase (PEP), which is a multi-subunit enzyme homologous to bacterial RNA polymerases. PEP consists of the plastid-encoded α, β, β', and β'' core-subunits and the nuclear-encoded sigma factor required for promoter recognition. Both polymerases need additional, yet unknown transcriptional factors (TF) for their correct function. Based on Liere *et al.*, 2011.

1.2 Regulation of organellar transcription

Advanced sensory systems allow higher plants to adjust their transcription in response to several exogenous and endogenous stimuli (Figure 2). Typical exogenous signals include light, mechanical forces, temperature, soil nutrients and humidity as well as presence of pathogens. Endogenous signals range from growth and developmental regulators to metabolites and defense signals (Gilroy and Trewavas, 2001). Regulation of organellar transcription by light and by the plant hormone cytokinin will be presented in more detail.

1.2.1 Light

Many important processes in higher plants are light-regulated including seedling photomorphogenesis, phototropism, chloroplast development, germination, circadian rhythms, flowering, and shade avoidance (Chen *et al.*, 2004; Franklin *et al.*, 2005; Jiao *et al.*, 2007; Franklin and Quail, 2010). Specific light qualities are required to modulate many processes in plants and plant cells. While red light controls processes such as seed germination, de-etiolation, shade avoidance and flowering (Casal *et al.*, 2003; Franklin and Quail, 2010), blue light is generally essential for the regulation of stomatal opening, inhibition of hypocotyls elongation, phototropism, opening of apical hook, and chloroplast movement

(Banerjee and Batschauer, 2005; Yu *et al.*, 2010). In addition, green light seems to be involved in controlling early developmental processes and is assumed to act antagonistically to blue light responses (Folta and Maruhnich, 2007). Furthermore, it was shown that certain light qualities mediated *via* specific photoreceptors strongly effect the expression of various genes in higher plants (Chun *et al.*, 2001; Thum *et al.*, 2001).

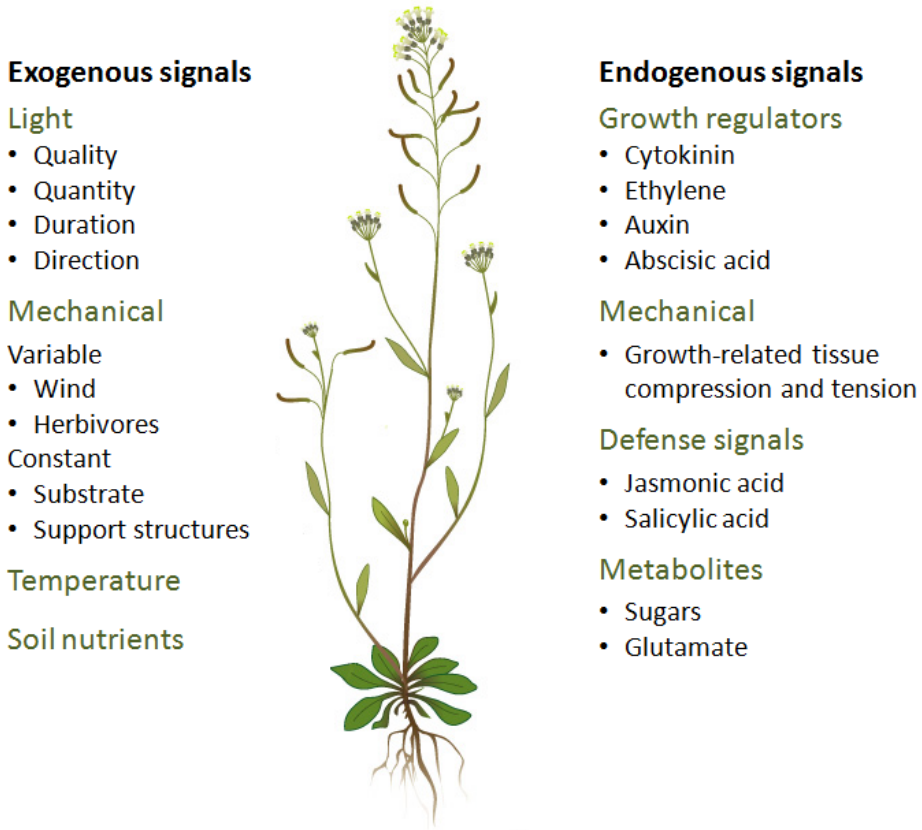

Figure 2: External and internal signals which might affect organellar transcription.

Plant growth and development in higher plants are regulated in response to a wide range of external and internal signals. The modulation of organellar transcription is an appropriate mechanism to adjust plant responses to changed growth conditions. Based on Gilroy and Trewavas, 2001.

1.2.1.1 Light perception

As sessile organisms, plants have evolved a number of different photoreceptors to perceive and respond to changing light conditions in their environment (Chory, 2010). At least twelve photoreceptors are known from *Arabidopsis* including five phytochromes (Smith, 2000; Franklin and Whitelam, 2004), two cryptochromes (Lin and Shalitin, 2003; Li and Yang, 2007), two phototropins (Briggs and Christie, 2002; Christie, 2007; Inoue *et al.*, 2008), and three Zeitlupe-like proteins (Imaizumi *et al.*, 2003; Ulm and Nagy, 2005; Briggs, 2007; Kim *et al.*, 2007). The diverse photoreceptors are defined by the color of light they predominately

absorb. Most important photoreceptors include the red light absorbing phytochromes (Quail *et al.*, 1995) and the blue light absorbing cryptochromes (Cashmore *et al.*, 1999; Lin and Shalitin, 2003). No specific photoreceptor for green light is known, but some of the green light responses are mediated *via* cryptochromes (Folta and Maruhnich, 2007).

Phytochromes possess several functions in plant development such as the control of germination, stem elongation, leaf expansion, and photomorphogenesis (Quail, 2010). They are encoded by a small multigene family (Mathews and Sharrock, 1997), which consists of five members in *Arabidopsis* with PHYA and PHYB being the most prominent (Smith, 2000; Franklin and Whitelam, 2004; Chen *et al.*, 2004). It was shown that the transcription of early responding genes in *Arabidopsis* under red and far-red light is mainly under control of PHYA (Tepperman *et al.*, 2001; Tepperman *et al.*, 2006). Furthermore, there are two sub-groups of phytochromes: type I phytochromes (PHYA) are photo-labile, while type II phytochromes (PHYB-PHYE) are photo-stable (Hirschfeld *et al.*, 1998). In general, phytochromes exist in two photoreversible isomeric forms: Pr (r; red) absorbs red light (660 nm) and Pfr (fr; far red) absorbs far-red light (730 nm). Red light leads to a reversible conversion of inactive Pr into active Pfr (Quail, 2002). This is followed by a nuclear translocation of Pfr where it binds to transcription factors for direct regulation of nuclear transcription (Chen *et al.*, 2004; Jiao *et al.*, 2007). *Vice versa*, absorption of far-red light promotes the conversion of the active Pfr form into the inactive Pr form.

Blue and UV-A light (340-520 nm) are sensed by phototropins, Zeitlupe-like proteins and cryptochromes. While the first two mainly mediate movement processes, cryptochromes regulate de-etiolation, photomorphogenesis, and flowering (Lin and Todo, 2005; Christie, 2007; Li and Yang, 2007; Demarsy and Fankhauser, 2009). For example, it was also shown that cryptochromes are involved in regulation of early blue light induced gene expression (Ohgishi *et al.*, 2004). *Arabidopsis* encodes genes for three different cryptochromes (*cry1-cry3*). While CRY1 and CRY2 act as blue light receptors in higher plants, it is still unclear if CRY3 functions as a photoreceptor (Kleine *et al.*, 2003). The photo-stable CRY1 regulates the transition from dark to light development under high light intensities, whereas the photo-labile CRY2 controls photoperiodic flowering in response to low light intensities (Lin, 2002). Interestingly, the predominantly red light perceiving PHYA is also able to absorb blue light (Casal and Mazzella, 1998; Neff and Chory, 1998; Poppe *et al.*, 1998).

Perception of light and regulation of light responses mediated by photoreceptors involve complex pathways (Figure 3). Therefore, many key transcription factors that serve as signal integration points are located in the light signaling networks downstream of photoreceptors such as phytochromes and cryptochromes (Jiao *et al.*, 2007). One of the key players is LONG HYPOCOTYL 5 (HY5), a basic leucine zipper (bZIP) transcription factor (Koornneef *et al.*, 1980; Oyama *et al.*, 1997; Ang *et al.*, 1998; Ulm *et al.*, 2004). Absent in darkness, it accumulates rapidly upon exposure to light and regulates the transcription of light-responsive genes (Ang *et al.*, 1998; Chattopadhyay *et al.*, 1998; Osterlund *et al.*, 2000). Lee *et al.* (2007) analyzed the genomic binding sites of HY5, which revealed its role as a major high hierarchical regulator in plant development. Furthermore, HY5 promotes photomorphogenesis under red, far-red and blue light conditions (Lau and Deng, 2010).

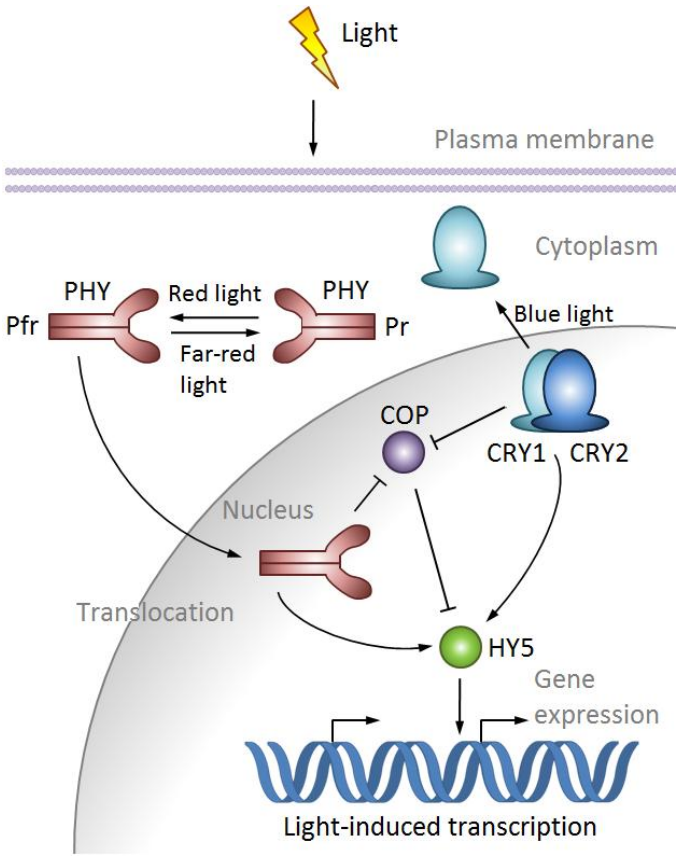

Figure 3: Simplified model of the light signaling pathway in *Arabidopsis thaliana*.

Cryptochromes and phytochromes account for the perception of light signals. Light conditions with a relatively high red:far-red ratio turn phytochrome from the inactive, cytoplasma-localized Pr form to the active, nuclear-localized Pfr form. Blue light exposure triggers the photoactivation of CRY1, while CRY2 remains in the nucleus. HY5 is a key transcription factor located downstream of photoreceptors to serve as a signal integration point. The COP transcription factor acts as repressor of HY5 and is inhibited by PHY and CRY. COP: constitutive photomorphogenic protein, CRY: cryptochrome, HY5: long hypocotyl 5, PHY: phytochrome, r: red; fr: far-red. Based on Jiao *et al.*, 2007.

1.2.1.2 Light and plastidial transcription

Light acts as an environmental signal to adjust plant growth and development (Casal *et al.*, 2004; Jiao *et al.*, 2007), but also plays an important role in activating the transcription of plastid and nuclear encoded genes involved in photosynthesis (Thompson and White, 1991; Rapp *et al.*, 1992; Christopher and Mullet, 1994; Mayfield *et al.*, 1995; Terzaghi and Cashmore, 1995; Link, 1996; Pfannschmidt *et al.*, 1999a,b; Tsunoyama *et al.*, 2002; Mochizuki *et al.*, 2004; Tsunoyama *et al.*, 2004; Granlund *et al.*, 2009). For instance, it was shown that both red and blue light regulate the expression of photosynthesis-associated, nuclear-encoded proteins such as CAB and RbcS (Fluhr and Chua, 1986; Karlin-Neumann *et al.*, 1988; Reed *et al.*, 1994; Mazzella *et al.*, 2001, Tyagi and Gaur, 2003). Light signals can even interact with plastid signals to ensure efficient chloroplast biogenesis (Ruckle *et al.*, 2007; Larkin and Ruckle, 2008).

Light-dependent transcription of plastid genes in leaves has been widely studied before (Greenberg *et al.*, 1989; Schrubar *et al.*, 1990; Klein and Mullet, 1990; Baumgartner *et al.*, 1993; Du Bell and Mullet, 1995; Hoffer and Christopher, 1997; Satoh *et al.*, 1997; Shiina *et al.*, 1998; Baena-Gonzalez *et al.*, 2001; Chun *et al.*, 2001; Nakamura *et al.*, 2003; Tepperman *et al.*, 2006; Dhingra *et al.*, 2006). Well-known examples of light-induced plastid genes are *psbA*, *psbD-psbC*, *petG*, *rbcL*, and *atpB* (Klein *et al.*, 1988; Haley and Bogorad, 1990; Klein and Mullet, 1990; Sexton *et al.*, 1990; Isono *et al.*, 1997). The transcription of the *psbD* gene, which encodes the D2 photosystem II reaction center protein, is activated by blue light. Responsible for the light-induced activation is the *psbD* blue light responsive promoter (BLRP; Sexton *et al.*, 1990). This promoter is found in the upstream region of the *psbD* gene of various plant species (Christopher *et al.*, 1992; Wada *et al.*, 1994; Allison and Maliga, 1995; Kim and Mullet, 1995; To *et al.*, 1996; Hoffer and Christopher, 1997; Kim *et al.*, 1999; Thum *et al.*, 2001). The nuclear-encoded sigma factor 5 (SIG5) was shown to be responsible for the blue light-induced activation of BLRP in *Arabidopsis* (Tsunoyama *et al.*, 2002, 2004; Mochizuki *et al.*, 2004; Nagashima *et al.*, 2004; Onda *et al.*, 2008). Chun *et al.* (2001) showed that blue light is also mainly responsible for the light-induced activation of chloroplast transcription as well as transcription of *psbA* and *rbcL* in *Arabidopsis* and tobacco. Both signal transduction pathways are assumed to involve reception of blue light by cryptochromes and phytochrome A (Chun *et al.*, 2001; Thum *et al.*, 2001; Mochizuki *et al.*, 2004).

1.2.2 Phytohormones

Phytohormones are small extracellular signal molecules, which can be easily transported through the entire plant. Hormones can act on nearby and distant cells and even low concentrations can result in significant effects (see review by Davies, 2004). Most phytohormones are derivatives of purines, amino acids, fatty acids or belong to the isoprenoid group (Figure 4).

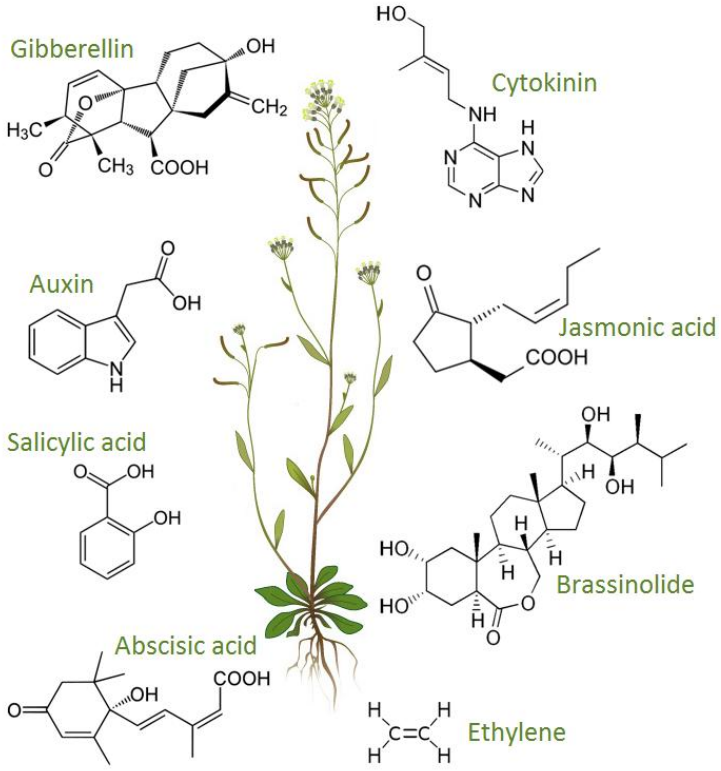

Figure 4: Different phytohormones regulate all aspects of plant growth and development.

Most prominent members of the phytohormone family in *Arabidopsis thaliana* are abscisic acid (ABA), indole-3-acetic acid (IAA or auxin), brassinosteroids (BRs), cytokinin, gibberellic acid (GA), ethylene, jasmonic acid (JA) and salicylic acid.

Prominent classic plant hormones are auxin, ethylene, cytokinin, gibberellins and abscisic acid (see reviews by Zhao, 2010; Lin *et al.*, 2009; Sakakibara, 2006; Razem *et al.*, 2006). Other identified plant growth regulators with characteristics of phytohormones include brassinolides, salicylic acid and jasmonic acid (see reviews by Asami *et al.*, 2005; Chen *et al.*, 2009; Gfeller *et al.*, 2010). As part of a coordinated network, plant hormones coordinate growth, development and responses to external stimuli. These processes are also influenced by various factors like light quality to mediate environmental changes (Weiler, 2003;

Vandenbussche *et al.*, 2007; Lau *et al.*, 2010). Phytohormone effects depend, among other things, on their site of action, concentration and plant developmental stage. In addition, the ratio of hormones plays a major role for their functionality, because different hormones often work in tandem. For example, early reports of Skoog and Miller (1957) revealed that shoot and root development is affected by the ratio of auxin and cytokinin.

1.2.2.1 Cytokinin

Discovered more than fifty years ago, cytokinins are a class of plant hormones, which showed the ability to trigger plant cell division *in vitro* (Miller *et al.*, 1955; Miller *et al.*, 1956). Cytokinins are adenine derivatives carrying either an isoprene-derived or an aromatic side chain at the N^6-position (see reviews by Mok and Mok, 2001; Sakakibara, 2006). These hormones occur either bound to certain tRNAs or as free cytokinins (Haberer and Kieber, 2002). Isopentenyladenine (iP), zeatin (Z) and dihydrozeatin (DZ) are the most abundant natural occurring isopenoid cytokinins, while aromatic cytokinin such as 6-benzyladenine (BA) are only found in selected plant species (Strnad, 1997; Sakakibara, 2006). The distribution of the various cytokinins differs significantly within plant species, tissues and developmental stage (Haberer and Kieber, 2002).

Cytokinins affect numerous aspects of development and physiology. For example, cytokinin is important for seed germination, leaf senescence, control of shoot and root meristem activity, photomorphogenesis and the flower/fruit development (Werner and Schmülling, 2009). Increased cytokinin levels improve resistance against several environmental stress factors such as drought, salts, cold- and heat-treatment, heavy metals and certain pathogens (see overview by Székács *et al.*, 2000). Chloroplasts are among the main targets of cytokinin action. Early experiments by Richmond and Lang (1957) showed that cytokinins are able to delay the loss of leaf chlorophyll during leaf senescence. Nearly fifty years later, Brenner *et al.* (2005) identified among the genes that responded early to cytokinin treatment in *Arabidopsis* the plastidial genes *petA*, *psbG*, *ycf10*, *ycf5* and *matK*. Cytokinins also play a major role in chloroplast differentiation (Chory *et al.*, 1994; Kusnetsov *et al.*, 1994).

1.2.2.2 Cytokinin reception pathway

Cytokinin signaling resembles the common bacterial two-component signaling systems, but is quite more complex (Figure 5; see reviews by To and Kieber, 2008; Santner *et al.*, 2009). Cytokinin signals are perceived by members of the histidine kinase (AHK) receptor family. Three different AHK plasma membrane receptors exist in *Arabidopsis*: AHK2, AHK3, and AHK4/CRE1/WOL (see review by Heyl *et al.*, 2011). The perception of cytokinin leads to a phosphorylation of histidine phosphotransfer proteins (AHP), which in turn are translocated to the nucleus and further transfer phosphates to response regulator proteins (ARR; Suzuki *et al.*, 2002).

There are two types of response regulators: 10 type-A regulators which are composed solely of a receiver domain (Brandstatter and Kieber, 1998; D'Agostino and Kieber, 1999; Imamura *et al.*, 1998) and 11 type-B regulators which have an additional output domain fused to the receiver (Kiba *et al.*, 1999; Hwang and Sheen, 2001; Sakai *et al.*, 2000). The phosphorylation of the type-B regulators leads to the activation of their output domain and to the transcriptional induction of cytokinin-induced genes, including those encoding type-A regulators (Hwang and Sheen, 2001). The type-A regulators act as repressors of cytokinin signaling *via* feedback regulatory mechanisms, whereas type-B regulators interact with various effectors to alter cellular functions (Cytokinin Response Factors, CRF; Hwang and Sheen, 2001; Mason *et al.*, 2004; Rashotte *et al.*, 2006). Recently, it was shown that a specific CRF domain defines cytokinin response factor proteins in higher plants (Rashotte and Goertzen, 2010).

A large number of cytokinin-regulated genes are present in *Arabidopsis thaliana* (Rashotte *et al.*, 2003; Peng *et al.*, 2009). In addition, *Arabidopsis thaliana* possesses cytokinin oxidase/dehydrogenase enzymes (CKX), which inactivate cytokinins irreversibly in a single enzymatic step (Mok and Mok, 2001). Werner *et al.* (2003) engineered cytokinin–deficient transgenic *Arabidopsis* plants that overexpress members of the CKX gene family to analyze cytokinin function in the shoot and root meristem activity. These transgenic plants had strongly decreased cytokinin contents compared to wild-type plants.

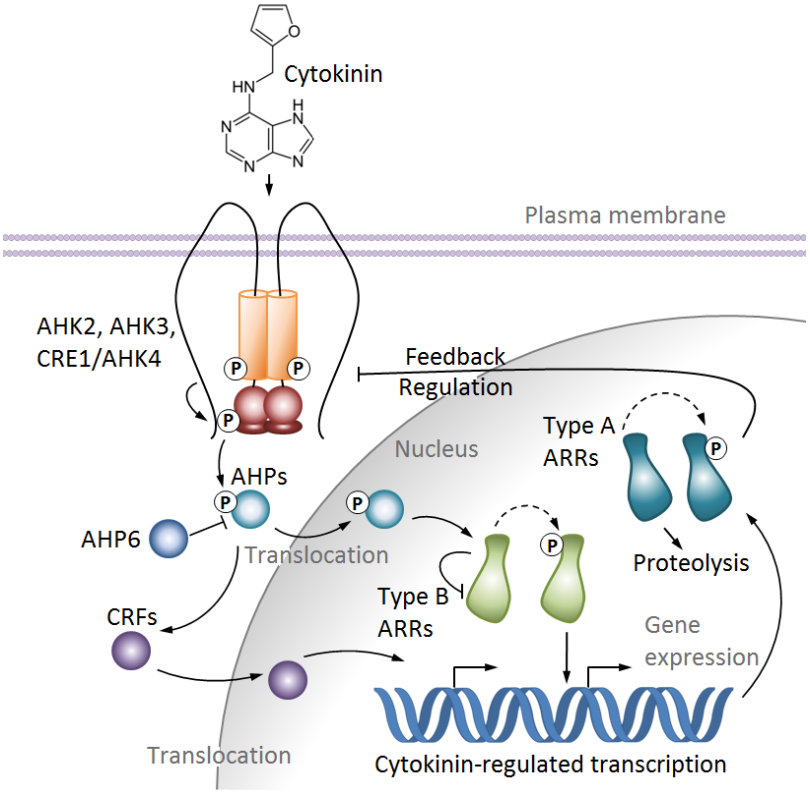

Figure 5: Schematic representation of the cytokinin signaling pathway in *Arabidopsis thaliana*.

Phosphorelay events mediate the hormone signaling from cytokinin receptors (AHK2, AHK3 and CRE1/AHK4) *via* AHP proteins to type-B response regulators including ARR1, which co-activate cytokinin-regulated gene transcription. The CRF proteins are also activated by cytokinin. AHK: Arabidopsis Histidine Kinase, AHP: Arabidopsis Histidine Phosphotransfer protein, ARR: Arabidopsis Response Regulator, CRF: Cytokinin Response Factors. Based on Santner *et al.*, 2009.

1.2.2.3 Cytokinin and chloroplasts

Cytokinins are involved in the control of chloroplast biogenesis and function. Hormone-regulated processes include chloroplast enzyme activities, pigment accumulation and the rate of photosynthesis (see overview by Zubo *et al.*, 2008). Exogenously applied cytokinins delayed senescence of detached leaves (Romanko *et al.*, 1969; Zubo *et al.*, 2008). Interestingly, many enzymes for cytokinin biosynthesis as well as some cytokinins are present in chloroplasts (Benková *et al.*, 1999; Kasahara *et al.*, 2004; Polanská *et al.*, 2007). Chloroplasts are also involved in the biosynthesis of abscisic acid, which acts as a cytokinin antagonist (Khokhlova *et al.*, 1978; Koiwai *et al.*, 2004). Cytokinin effects on the expression of nuclear genes encoding chloroplast proteins may at least in part account for plastidial responses (Chory *et al.*, 1994; Kusnetsov *et al.*, 1994; Hutchison and Kieber, 2002; Rashotte *et al.*, 2003; Brenner *et al.*, 2005; Kiba *et al.*, 2005).

Recent data show that the application of cytokinin increased the transcription of some plastidial genes such as *petA*, *psbA*, *matK*, *rrn16*, and *petD* in leaves of barley, tobacco and *Arabidopsis thaliana* (Zubo *et al.*, 2008; Brenner *et al.*, 2005; Hertel, 2009). For example, total chloroplast transcription in barley was stimulated by a plastidial cytokinin-binding protein (zeatin-binding protein; ZBP_{Chl}) in an age-dependent manner (Kulaeva *et al.*, 2000; Lyukevich *et al.*, 2002). Many studies indicate a role of cytokinin in the regulation of plastidial transcript levels (Lerbs *et al.*, 1984; Stabel *et al.*, 1991; Masuda *et al.*, 1994; Hande and Jayabaskaran, 1996; Kasten *et al.*, 1997). Cytokinin was able to activate chloroplast transcription in *Arabidopsis* and in tobacco (Hertel, 2009). The stabilization of transcripts occurred very fast after 15 min of incubation with cytokinin, as indicated by increasing steady state levels. Chloroplast transcription however, responded much slower to the hormonal stimulus showing increased activity after two hours in *Arabidopsis* and three hours in tobacco.

Microarray analysis showed that a high percentage of cytokinin-regulated genes are involved in transcriptional control or are associated with developmental processes (Brenner *et al.*, 2005). Furthermore, transcripts of five plastid genes (*petA*, *psbG*, *ycf10*, *ycf5*, *matK*) were up-regulated early on, indicating either a rapid transfer of the signal to the chloroplasts or a direct, plastidial perception of the cytokinin signal. These results suggest that cytokinin might act under certain conditions on transcript accumulation, modification of transcripts, and translation in plastids (Brenner *et al.*, 2005).

1.3 Aim of this work

Several studies describe the effects of light and/or hormones on chloroplast development and function (see 1.2.1 and 1.2.2). Not much is known though about regulation of organellar gene expression in response to light signals or exogenous application of cytokinin. However, the molecular mechanisms how the plant hormone cytokinin and different light qualities unfold their effects on organellar gene transcription are still under investigation.

Therefore, quantitative real-time PCR analyses was applied in the present study to gain more information about light-induced expression of organellar RNA polymerases, accumulation of transcripts of genes encoding the nuclear-encoded organellar phage-type RNA polymerase (*RpoT*) and subunits of the plastidial eubacterial-type RNA polymerase (*rpoB* operon). To learn more about photoreceptors and light-related pathways involved in light-induced gene expression, wild-type seedlings and different photoreceptor mutants will be analyzed under selected light qualities.

To gain more information about the signaling pathways involved in cytokinin action in chloroplasts, activation of transcription of plastidial genes will be analyzed in several cytokinin-related mutants by run-on transcription assays in comparison to wild-type seedlings. Furthermore, the influence of cytokinin on cellular parameters such as chloroplast size, number, and DNA content will be studied. For studying the importance of sigma factors in cytokinin-dependent regulation of chloroplast transcription, accumulation of plastidial transcripts will be analyzed for activation by cytokinin in sigma factor mutants by run-on assays and quantitative real-time PCR in comparison to wild-type plants.

2 Materials and Methods

2.1 Materials

Chemicals and biochemicals were generally purchased from Biozym, ICN Biomedical, Roth, Merck, Serva, Sigma-Aldrich and Qiagen, unless specified otherwise. Ultrapure water was obtained from a USF Purelab Plus system. Sterilization of solutions, buffers and hardware, as well as inactivation of genetically modified material was carried out in the Varioklav 75 S steam sterilizer (Thermo Scientific) at 120 °C and 55 kPa for 20 min.

2.1.1 Providers

AppliChem	AppliChem GmbH, Darmstadt, Germany
Applied Biosystems	Applied Biosystems, Weiterstadt, Germany
Ambion	Ambion, Inc., Austin, TX, USA
Amersham Biosciences	Amersham Biosciences Europe GmbH, Freiburg, Germany
BD Biosciences	BD Biosciences, Franklin Lakes, NJ, USA
Biometra	Biometra GmbH, Göttingen, Germany
Bio-Rad	Bio-Rad Laboratories, Richmond, VA, USA
Biozym	Biozym Diagnostik GmbH, Hameln, Germany
Braun	Braun GmbH, Kronberg, Germany
Calbiochem	Calbiochem Merck Biosciences GmbH, Schwalbach, Germany
CLF	CLF Plant Climatics GmbH, Wertingen, Germany
Colgate-Palmolive	Colgate-Palmolive Company, New York, NY, USA
Duchefa	Duchefa Biochemie B.V., Haarlem, The Netherlands
DuPont	DuPont de Nemours GmbH, Bad Homburg, Germany
Epicentre	Epicentre Biotechnologies, Madison, WI, USA
Eurogentec	Eurogentec, Seraing, Belgium
Everlight	Everlight Electronics, Taipeh, Taiwan
Fermentas	Fermentas GmbH, St. Leon-Rot, Germany
Franz Eckert GmbH	Franz Eckert GmbH, Waldkirch, Germany
GE Healthcare	GE Healthcare Europe GmbH, Freiburg, Germany
Heraeus	Heraeus, Hanau, Germany
ICN	ICN Biochemicals Inc., Ohio, Germany
Invitrogen	Invitrogen GmbH, Karlsruhe, Germany
Jenoptik	Jenoptik L.O.S. GmbH, Jena, Germany
Macherey-Nagel	Macherey-Nagel, Düren, Germany
Metabion	metabion international AG, Martinsried, Germany
Millipore	Millipore Corp., Bedford, USA
Nalgene	Nalgene®Labware, Rochester, NY, USA
Operon	Operon Biotechnologies GmbH, Köln, Germany
peqLab	peqLab Biotechnologie GmbH, Erlangen, Germany
Perkin Elmer	Perkin Elmer LAS GmbH, Rodgau, Germany
Philips	Philips Electronics, Amsterdam, The Netherlands
Pierce	Pierce, Rockford, IL, USA
Promega	Promega Corp., Madison, WI, USA
Qiagen	Qiagen, Hilden, Germany
Roche	Roche Diagnostics GmbH, Mannheim, Germany

Roth	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Serva	Serva Feinbiochemika, Heidelberg, Germany
Sorvall	Kendro Laboratory Products GmbH, Langenselbold, Germany
Sigma-Aldrich	Sigma-Aldrich Corporation, St. Luis, MO, USA
SMB GmbH	Services in Molecular Biology GmbH, Berlin, Germany
Stratagene	Stratagene, La Jolla, CA, USA
Thermo Scientific	Thermo Scientific LED GmbH, Langenselbold, Germany
USF	USF, Seral Reinstwassersysteme GmbH, Germany
Whatman	Whatman Paper, Maidstone, UK
Zeiss	Carl Zeiss MicroImaging GmbH, Jena, Germany

2.1.2 Plant material

Arabidopsis thaliana wild-type plants were grown from seeds of the ecotype Columbia (Col-0) and Landsberg *erecta* (L*er*). Seeds of photoreceptor mutants (Table 1) were kindly provided by Prof. Hellmann (Freie Universität Berlin) and Prof. Batschauer (Philipps-Universität Marburg). Seeds of cytokinin-related mutants (Table 2) were kindly provided by Dr. Riefler and Prof. Schmülling (Freie Universität Berlin). Seeds of sigma factor mutants (Table 3) were ordered *via* GABI-Kat and NASC, while *sig2* and *sig4* mutants were kindly provided by Dr. Schweer (Ruhr-Universität Bochum).

Table 1: Employed photoreceptor mutant plants.

name	mutation	mutant denotation	ecotype background
phyA	knockout of the gene *phyA*, leading to plants lacking the photoreceptor phytochrome A	*phyA-201*	L*er*
phyB	knockout of the gene *phyB*, leading to plants lacking the photoreceptor phytochrome B	*phyB-5*	L*er*
phyA/phyB	knockout of the genes *phyA* and *phyB*, leading to plants lacking the photoreceptors phytochrome A and B	*phyA-201/phyB-5*	L*er*
cry1	knockout of the gene *cry1*, leading to plants lacking the photoreceptor cryptochrome 1	*cry1-1*	L*er*
cry2	knockout of the gene *cry2*, leading to plants lacking the photoreceptor cryptochrome 2	*fha-1*	L*er*
cry1/cry2	knockout of the genes *cry1* and *cry2*, leading to plants lacking the photoreceptors cryptochrome 1 and 2	*cry1-1/fha-1*	L*er*
hy5	knockout of the gene *hy5*, leading to plants lacking the transcription factor HY5	*hy5*	L*er*

Table 2: Employed cytokinin-related mutant plants.

name	mutation	mutant denotation	ecotype background
cre1	knockout of the gene *cre1*, leading to plants lacking the cytokinin receptor histidine kinase 1	*cre1-2*	Col-0
ahk2	knockout of the gene *ahk2*, leading to plants lacking the cytokinin receptor histidine kinase 2	*ahk2-5*	Col-0
ahk3	knockout of the gene *ahk3*, leading to plants lacking the cytokinin receptor histidine kinase 3	*ahk3-7*	Col-0

ahk2/cre1	knockout of the genes *ahk2* and *cry1*, leading to plants lacking the cytokinin receptors histidine kinase 2 and 1	*ahk2-5/cre1-2*	Col-0
ahk3/cre1	knockout of the genes *ahk3* and *cry1*, leading to plants lacking the cytokinin receptors histidine kinase 3 and 1	*ahk3-7/cre1-2*	Col-0
ahk2/ahk3	knockout of the genes *ahk2* and *ahk3*, leading to plants lacking the cytokinin receptors histidine kinase 2 and 3	*ahk2-5/ahk3-7*	Col-0
ARR1	fusion of the B-type response regulator ARR1 to the repressor motif SRDX, increase resistance to cytokinin	*35S::ARR1-SRDX*	Col-0
CKX1	leading to cytokinin-deficient transgenic plants	*35SAth::CKX1*	Col-0

Table 3: Employed sigma factor mutant plants.

name	mutation	mutant denotation	ecotype background
sig1	knockout of the gene *sig1*, leading to plants lacking the sigma factor 1	*sig1-1*	Col-0
sig2	knockout of the gene *sig2*, leading to plants lacking the sigma factor 2	*sig2-1*	Col-0
sig3	knockout of the gene *sig3*, leading to plants lacking the sigma factor 3	*sig3-4*	Col-0
sig4	knockout of the gene *sig4*, leading to plants lacking the sigma factor 4	*sig4-1*	Col-0
sig5	knockout of the gene *sig5*, leading to plants lacking the sigma factor 5	*sig5-1*	Col-0
sig6	knockout of the gene *sig6*, leading to plants lacking the sigma factor 6	*sig6-2*	Col-0

2.1.3 Oligonucleotides

Oligonucleotides were provided by Sigma-Genosys (Sigma-Aldrich) or Operon. Sequences of oligonucleotides are specified in the chapters, respectively.

2.1.4 Software

Primers for quantitative real-time PCR were designed using the ProbeFinder Software of the Universal ProbeLibrary Assay Design Center (Roche Applied Science, https://www.roche-applied-science.com/sis/rtpcr/upl). Design of text and graphics was carried out using Microsoft Office Word 2007, Microsoft Office Excel 2007, and Microsoft Office Power Point 2007. Statistical significance of data was investigated using GraphPad QuickCalc (GraphPad Software Inc, San Diego, USA, http://www.graphpad.com/quickcalcs/index.cfm). Radioactive signals were detected and quantified by scanning using Molecular Imager FX and Quantity One software, version 4.6.2 (Bio-Rad). Quantitative real-time PCR data were analyzed using the Sequence Detection Software v1.4 (Applied Biosystems). Flow cytometric data were analyzed using CELL QUEST Software v3.3 (BD Biosciences).

2.2 Methods

2.2.1 Surface sterilization of *Arabidopsis thaliana* seeds

Arabidopsis thaliana seeds were incubated in sterilization solution and shaken gently. After seven minutes they were harvested in a microcentrifuge and the supernatant was discarded. Seeds were then washed five times in sterile water. After the last washing step seeds were transferred to a petri dish with sterilized SEA medium.

sterilization solution: 32 % (v/v) DanKlorix (Colgate-Palmolive); 0.8 % (w/v) N-lauryl-sarcosine

2.2.2 Plant growth

Seedlings for light induction analyses (red, blue and green light)

Surface-sterilized *Arabidopsis thaliana* (ecotype Landsberg *erecta*) seeds were sown on sterilized SEA medium containing sucrose (10 g/L). Plants were grown in complete darkness at 23 °C. After seven days, a fraction of the seedlings was harvested directly as dark controls. The remaining etiolated seedlings were put into light of the respective wavelength and harvested after one, four, six, twelve and twenty-four hours. Different light regimes were achieved by placing LED arrays in a darkened chamber. Illumination for all experiments was obtained with light-emitting diode blue light (470 ± 35 nm; 4 µmol m^{-2} s^{-1}) lamps (264-7SUBC/C470/S400-A4; Everlight), red light (631 ± 20 nm; 11 µmol m^{-2} s^{-1}) lamps (7343USRC/TL; Everlight) and green light (530 ± 35 nm; 3 µmol m^{-2} s^{-1}) lamps (246-7SUGC/S400-A5; Everlight).

SEA medium: 0.44 % (w/v) MS basal medium (M0222; Duchefa); 0.05 % (w/v) MES in ultrapure water; 1.5 % (w/v) plant agar (P1001.1000; Duchefa); pH 5.7

Seedlings for light induction analyses (white light)

Surface-sterilized *Arabidopsis thaliana* (ecotype Landsberg *erecta*) seeds were sown on sterilized SEA medium containing sucrose (10 g/L). Plants were grown in complete darkness at 23 °C. After seven days, part of the seedlings was harvested directly as dark controls. The remaining etiolated seedlings were put into the light and harvested after one, four, six and twelve hours. Light intensity was set at ~ 270 µmol m^{-2} s^{-1} (Lamp Master HPI-T Plus 400W E40; Philips).

Seedlings for cytokinin experiments (sown on net)

Arabidopsis thaliana (ecotype Columbia Col-0 and Landsberg *erecta*) seeds were sown on top of polyamide-nets (mesh size 500 µM; Franz Eckert GmbH) laid out on a vermiculite/soil-mix (1:1) in petri dishes. Plants were grown at 23 °C under illumination of 270 µmol m^{-2} s^{-1} from luminescent tubes (Lamp Master HPI-T Plus 400W E40; Philips) with a 16-h photoperiod. After twelve days seedlings were cut and washed twice in water to remove residual soil particles. The seedlings were incubated in water under continuous illumination of 270 µmol m^{-2} s^{-1} for 24 h. Subsequently, the seedlings were transferred to water or a solution of the synthetic cytokinin 6-benzyladenin (BA; 2.2 x 10^{-5} M; ICN) and kept for 6 h under the same light conditions.

Seedlings for cytokinin experiments (sown on medium)

Surfaced-sterilized *Arabidopsis thaliana* (ecotype Columbia Col-0) seeds were sown on sterilized Murashige and Skoog (MS) medium. For cytokinin treatment, sterilized seeds were sown on MS plates supplemented with 5 mM BA or without BA and grown for 11 days. Plants were grown at 23 °C under illumination of 270 µmol m^{-2} s^{-1} from luminescent tubes (Lamp Master HPI-T Plus 400W E40; Philips) with a 16-h photoperiod.

MS medium:	0.44 % (w/v) MS basal medium (M0222; Duchefa); 0.05 % (w/v) MES in ultrapure water; 1 % (w/v) plant agar (P1001.1000; Duchefa); pH 5.7

2.2.3 Microscopy

For observation of chloroplasts in *Arabidopsis* leaf cells, ten first leaves from ten days-old plants grown on MS plates were cut and solubilized in organelle isolation solution. Samples were analyzed using a light microscope (Axioskop; Zeiss) with an oil immersion objective (Plan-NEOFLUAR 100 x/1.30 Oil; Zeiss) or a 40 x objective (Plan-NEOFLUAR 40 x/0.75; Zeiss). For the determination of the diameter of chloroplasts at least 100 chloroplasts were analyzed and for the comparison of the number of chloroplast per mesophyll cell at least 17 cells were analyzed.

isolation solution:	0.33 M sorbitol; 50 mM HEPES (pH 7.6); 2 mM EDTA; 1 mM $MgCl_2$; 0.1 % BSA; 1% PVP-40; 5 mM ß-mercaptoethanol

2.2.4 Isolation of nucleic acids

2.2.4.1 Isolation of total DNA

Total DNA from *Arabidopsis* samples was isolated using the DNeasy® Plant Mini Kit (Qiagen) according to the manufacturer‗s protocol. The concentration of the DNA was determined spectrophotometrically using the Nanodrop® ND-1000 system (peqLab).

2.2.4.2 Isolation of total RNA

Total RNA from etiolated *Arabidopsis* samples was isolated using the RNeasy® Plant Mini Kit (Qiagen) with Buffer RLT according to the manufacturer‘s protocol. Total RNA from green tissue was isolated using the TRIzol® Reagent (Invitrogen) according to the manufacturer‘s protocol. RNA quality was controlled by denaturing agarose gel electrophoresis (see 2.2.5) and concentrations were quantified spectrophotometrically.

2.2.5 Analytical agarose gel electrophoresis of RNA

RNA samples were mixed with RNA loading dye, denatured at 95 °C for 10 min, incubated on ice for 5 min, and subsequently separated in a 1 % (w/v) agarose gel containing $^1/_{40}$ vol formaldehyde in 1x MEN running buffer. The voltage was set at 2.5 - 5 V/cm. RNA bands were subsequently visualized under UV-light excitation in the Gel Doc XR System (Bio-Rad).

10x MEN:	200 mM MOPS; 50 mM NaAc; 10 mM EDTA; pH 7.0 with NaOH
RNA loading dye:	1 ml formamide; 350 µl formaldehyde, 200 µl 10x MEN; 400 µl glycerol; 5 µl 0.5 M EDTA, pH 8.0; 10 µl 10 mg/ml EtBr; 2 mg bromophenol blue; 2 mg xylene cyanol; ultrapure water ad 2 ml

2.2.6 The reverse transcription of total RNA

QuantiTect® Reverse Transcription Kit (Qiagen) was used to eliminate remaining genomic DNA from the RNA samples and subsequently reverse-transcribe the RNA according to the manufacturer‘s protocol.

2.2.7 Quantitative real-time PCR with probes

Primer pairs for quantitative real-time PCR of cDNA samples were designed to yield amplification products of 70-100 bp. The PCR reactions were carried out in a 7500 Real-Time PCR System (Applied Biosystems) using the *TaqMan*® Fast Universal PCR Master Mix (Applied Biosystems) and the Universal Probe Library Set, *Arabidopsis* (Roche Applied Science) for detection according to the manufacturers protocols. Each reaction contained 50 ng cDNA, 1 µM of each primer (Table 4) and 100 nM of the particular probe. The cycle protocol consisted of an initial step at 95 °C for 10 min to activate the polymerase, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C.

To verify removal of genomic DNA from cDNA samples, a negative control (without addition of reverse transcriptase) was included for each reverse transcribed RNA sample. Each of the biological and technical replicates was analyzed in triplicates per experiment. In addition, no-template controls (NTC) were included for each primer pair. Data were analyzed using the Sequence Detection Software v1.4 (Applied Biosystems). All quantitations were normalized to the amount of nuclear *UBQ11* transcripts as internal standard using the ΔC_T method ($2^{(-\Delta C_T)}$ = relative amount of transcripts; $\Delta C_T = C_T^{\text{target}} - C_T^{\text{internal standard}}$).

Table 4: Primers used in quantitative real-time PCR analyses (Roche Applied Science, USA).

gene name	nucleotide sequence (5'→ 3')	position	probe #
RpoTm	ACAGAAATTGCGGCTAGGG GGCATATGTGGCATTTGGA	Chromosome I	6
RpoTmp	CGATGCCATTGAACAAGAGAT TGTTCCTTCATAGAAGTTTCATTTTC	Chromosome V	91
RpoTp	TTGCAGAAGTGAAAGACATCTGA ATCGACCGTGTTACCCTCTC	Chromosome II	21
UBQ11	CTTATCTTCGCCGGAAAGC GAGGGTGGATTCCTTCTGG	Chromosome IV	88
cab1	TGCTGCACTACTCAACCTCAA AAAGCTTGACGGCCTTACC	Chromosome I	52
elip1	TTGCCGAAGTCACCATCTC GCAAGTCGCTAAACTTTGTGC	Chromosome III	63
AIP	CGGTTTCGTACTTGGACCAG TTGGATGATCAAATCCAAACTCT	Chromosome IV	13
sig1	TCGCAGAAGAAAGTTAGAAATGC CCAGGGAGACCATTCAAAGA	Chromosome I	110
sig2	CGATGGTCCTTCCACTGAG CTGCTTCATCGCTTGTGAGA	Chromosome I	110
sig3	TCCCCATTCCCAAACAGA CACTAAAATACGTGGCCGAGA	Chromosome III	101

sig4	CGCATGACATTGCAGGAA TTCATGTGTTCCCTTTTCACC	Chromosome V	82
sig5	CAAGTTGATGCAACCTATGAAGG CGGCTATTTCAGCTTCCCTA	Chromosome V	103
sig6	AATCGTGGACTCAACTTTCAGG ACTTTTCATTAGCCCCATGC	Chromosome II	4
psbD	TGCGACCTTATAATGCAATCG GGAAGACAGAAACAAAAACAGCA	33146 33185	126
rpoB	TCTCGGTCCGAAAAGTGC CGGGAACCCCTGAATCTAA	24607 24658	154
psaA	ACACAACTGTCATTGTTCACACA GCAGCAGCACAACTATAGAG	40774 40816	138

2.2.8 Quantitative real-time PCR with SYBR® Green

Primer pairs were designed to yield amplification products of 70-100 bp. The PCR reactions were carried out in a 7500 Real-Time PCR System (Applied Biosystems) using the *Power* SYBR® Green PCR Master Mix (Applied Biosystems) for detection according to the manufacturers protocols. Each reaction contained 0.1 ng total DNA and 1 µM of each primer (Table 5). The cycle protocol consisted of an initial step at 50 °C for 2 min, than a step at 95 °C for 10 min, followed by 40 cycles of 15 s at 95 °C, 30 s at 60 °C and 45 s at 72 °C.

To verify the specificity of DNA amplification products a dissociation curve was added for each of the 96 wells by subjecting the samples to a heat-denaturation over a temperature gradient from 60 °C to 95 °C at 0.03 °C/s. Each of the biological replicates was analyzed in two technical repetitions and a triplicate was used for each sample. In addition, no-template controls were included for each primer pair. Data were analyzed using the Sequence Detection Software v1.4 (Applied Biosystems). All quantitations were normalized to the amount of the nuclear-encoded single-copy gene *RpoTm* (gDNA) as internal standard using the ΔC_T method ($2^{(-\Delta C_T)}$ = relative amount of transcripts; $\Delta C_T = C_T^{\text{target}} - C_T^{\text{internal standard}}$).

Table 5: Primers used in quantitative real-time PCR analyses (SYBR® Green).

gene name	nucleotide sequence (5'→ 3')	5' position
RpoTm	AGCCTGTGCGTAATGCTATTCA GCCATCTTATCAGCCGGTAACT	Chromosome I
clpP	TTGGTAATTGCTCCTCCGACT TATGCAATTTGTGCGACCC	70767 70693
psbA	AACTAAGTTCCCACTCACGACC CATCCGTTGATGAATGGCTAT‘	1063 1146

2.2.9 Detection of proteins by Western blotting

Protein samples prepared by homogenizing 7-d-old etiolated seedlings of *Arabidopsis* wild type and the phytochrome-deficient mutants were fractionated by SDS-PAGE (10 µg of total protein on a 7.5% PAA-gel) and blotted to a Hybond-C membrane (Amersham Bioscience). Samples were analyzed and the equal loading and transfer of proteins was monitored by staining the blot with Ponceau S (Sigma-Aldrich). The blot was probed with anti-Arabidopsis PHYA monoclonal antibody (Table 6). The PHYA antibody, Blocking Buffer I (AppliChem; no. A7099) and CrossDown Buffer (AppliChem; no. A6485) were kindly provided by Dr. Czarnecki (Humboldt Universität Berlin). Preparation of extracts from seedlings and immunochemical detection was carried out following the standard protocols as described in Sambrook and Russell (2001).

Table 6: Antisera.

antibody	properties	dilution	supplier
anti-phyA	raised against phytochrome A in *Arabidopsis*	1:2000	O. Czarnecki, HU Berlin
secondary antibody	anti-rabbit IgG-horseradish peroxidase conjugate	1:10000	Sigma-Aldrich

2.2.10 Blotting of chloroplast genes

Gene fragments were dotted onto nylon Hybond-N+ membrane (Amersham Bioscience). One µg of DNA of each gene fragment treated as described by Zubo and Kusnetsov (2008) was loaded onto the membrane in two replicates using a Bio-Dot apparatus (Bio-Rad). The gene-specific fragments used were kindly provided by Dr. Hertel and Dr. Zubo (Humboldt Universität Berlin), and are listed in Table 7.

Table 7: Chloroplast genes analyzed in run-on assays.

denotation	nucleotide sequence (5'→3')	5' position in ptDNA
atpB	AGGTCCTGTCGATACTCGCA	53022
	ATCTAAAGGATCTACCGCTGGATA	53766
atpF	GATTCTTTCGTTTACTTGGGTCAC	11544
	TTTAATATCCTCTGCTTTCGGTTATC	12428
atpH	TTTCTGCTGCTTCGGTTATTG	13275
	GCTAATGCTACAACCAGGCCATA	13479
ndhB	AATTTCTCAAACGAACCGCACTC	96389
	TCCTATTCATGGGGATTCCGTAA	97249
ndhI	GTCAACAAACCCTACGAGCTGC	119278
	TCAATTCGTGACGATCATAAGTGG	119649
petA	CATCCATTTCAAGTGCATATCC	61745
	CTTATTATCCCTCCTGCCGTAG	62300

petB	TAGTAAATATGTTCCTCCGCATGTC	75710
	GACGGCCGTAAGAAGAGGTAAT	76235
petD	TAGCTAAAGGTATGGGTCACAATTATTAC	77242
	AATCAAAAAGACGGTTGTCGC	77594
psaA	GCAGCAGCACCAACTATGAGA	40667
	GATCCTAAAGAAATACCGCTTCCTC	41170
psaB	CGGGTCATATGTATAGAACGAACTTTG	38234
	CAAGCCGAAATATCACAAGTACCAC	39092
psaC	ATTAGAAATGATACCTTGGGATGGAT	117392
	TGTTTCATGCCATAAATAAACTCGAAC	117536
psbA	ACTTCTGTTTTTATTATCGCATTCATTG-	515
	TCCATACCAAGGTTAGCACGG	1368
psbD	GTAGCGGCTATATTTCGATTCATCC	33260
	GCCATCCAAGCACGAATACCT	33702
psbE	TTCATTGCGGGCTGGTTATT	64161
	CAAAGGATCAAAACGGCCTGT	64289
psbK	TAAAAGGATTTTTGATTGAGTAAGTTCAAC	6915
	AAGAAAGAAAAGAGGTATTACGGGC	7160
rbcL	ATATCTTGGCAGCATTCCGAGTAACT	55157
	AGTATTTGCGGTGAATCCCCC	55955
accD	ATGGTTGGGATGAGCGTTCT	57223
	AAGTACCCGGATCAATCGAAA	57885
clpP	CCGACTAGGATAAAGGATGCTATTG	70690
	CCAAGAGGTTGATACCGAAATC	70908
rpoB	TATTATATGATAGCGGCAGGAAATT	24542
	ATAGGAGGATTCTTTCGCCACT	25372
rps4	ATCTTAGAAACCAATCACGCTCC	45314
	AAACCGACGCATTTCCTATCT	45779
rps8	GATCGACTAACATCACGGAAAGTATTG	80147
	TCTCGGTCTGTCATTATACCTTGA	80423
rps14	CCCGAAGGATGTGTCCAGATAG	37009
	AGAAGAAGAGGCAAAAATTGGAAAA	37212
rps16	TCGCACTAACCCTAAATCCTTACTC	5953
	CAAACTAGAGGAATGTTATGGTAAAACTTC	6205
rnn16	ATTGGGCGTAAAGCGTCTGTA	101522
	GTAACGACTTCGGGCATGG	102402
trnK 3'-intron	ACATCAAAATAAGATTGTACCGATCAG	4154
	TGACAACAGTGTATGGACCAAATATAA	4485
trnK 5'-intron	AGAAGCGAATCCACATACATAGAAATA	1657
	ATAAGGAACCAAAGAAATTGAGTTTTC	1884
trnL	AACGATCTCAAAAATGACGACC	47232
	GGGAGTAGAGCTGGGGATAGAG	47501
ycf1	AATTCGGTCGTTGTGGTCGG	109484
	TGCTAAATGCAGAGGCGCA	109665
ycf2	GATAGGAAGGGCTGTTGCACA	92223
	5GGGTCGAGGACTCCTTCTCC	92703
ycf5	TTAGTACCAGCTCTCCAGTCCC	114838
	ATAAAACCGATCAAAGCCACAA	115368
ycf10	TGGAATACTAGACAATGCGAAACTT	60855
	TACAAGTGACGGAGATACACGATT	61403

2.2.11 Chloroplast isolation

Arabidopsis thaliana seedlings (3-4g) were homogenized in 180 ml isolation buffer. The homogenate was squeezed through two layers of Miracloth (Calbiochem-Behring) and centrifuged at 2,000*g* for 6 min. The pellet was resuspended in 4 ml isolation buffer and fractionated in a 35%/70% discontinuous Percoll gradient by centrifugation at 6,500*g* for 15 min. Intact chloroplasts were collected at the interface between 35% and 70% Percoll, washed and resuspended in 0.5 ml isolation buffer. All procedures were performed at 4° C. The number of chloroplasts in the samples was determined by counting the organelles with a light microscope using a Fuchs-Rosenthal hemocytometer (Brown and Rickless, 1949). The chloroplasts were used for further run-on transcription.

percoll buffer:	15g PEG-8000; 2.5g BSA; 2.5g Ficoll; ß-mercaptoethanol-free isolation buffer ad 500 ml
isolation buffer:	5 mM ß-mercaptoethanol; 50 mM Tricine pH 8.0; 2 mM EDTA; 0.33 M sorbitol

2.2.12 Run-On Transcription Assay

Run-on transcription assays with $5x10^7$ lysed plastids were carried out in a 100 µl volume by the method of Mullet and Klein (1987) and modified as described by Zubo (2008). Transcription was performed for 10 min at 25 °C in transcription buffer. The reaction was stopped by the addition of an equal volume of stop buffer.

^{32}P-labeled transcripts were isolated from chloroplasts as described by Zubo and Kusnetsov (2008) and hybridized to plastid genes blotted on a nylon membrane in a blotting buffer. Radioactive signals were detected and quantified by scanning using the Molecular Imager FX and Quantity One software (Bio-Rad). Cytokinin effects on transcription were considered significant if the signals differed at least twofold from the water control. Every experiment was repeated at least two times.

transcription buffer:	50 mM Tris-HCl pH 8.0; 10 mM $MgCl_2$; 0.2 mM CTP, GTP and ATP; 0.01 mM UTP; 50 mCi [α-32P] UTP (Amersham); 20 units RNase-Inhibitor (Fermentas); 10 mM β-mercaptoethanol
stop buffer:	50 mM Tris-HCl pH 8.0; 25 mM EDTA; 5% sarcosyl
blotting buffer:	250 mM Na_2HPO_4; 7% SDS; 2.5 mM EDTA

2.2.13 Flow cytometric analysis of nuclear endo-polyploidy

Relative gene copy numbers of the chloroplast genes *psbA* and *clpP* were determined by quantitative real-time PCR (see 2.2.8). In addition, for the correct calculation of gene copies per cell, knowledge of nuclear ploidy level was required. Flow cytometric measurements and sorting of nuclear suspensions were carried out as described by Barow and Meister (2003) using a FACS Aria flow cytometer (BD Biosciences). The *C* values of about 10,000 nuclei were measured per leaf sample, using in total three independent leaf samples per experiment.

The mean *C* value was estimated as a weighted average using the formula $[(2n_{2C}) + (4n_{4C}) + (8n_{8C}) \ldots]/[n_{2C} + n_{4C} + n_{8C} \ldots]$, where n is the number of nuclei and *C* is the ploidy number (2C, 4C, 8C, …). Flow cytometric analysis was performed by Emilia Cincu (Humboldt Universität Berlin) and Dr. Fuchs (Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany).

3 Results

3.1 Analysis of light effects on the organellar gene expression

Higher plants are sessile organisms and therefore possess a wide number of photoreceptors for detection of different light qualities in their environment (Chen *et al.*, 2004; Chory *et al.*, 2010). Photoreceptor mutations lead to distinct phenotypes in *Arabidopsis* (Figure 6; Koornneef *et al.*, 1980, 1991; Ahmad and Cashmore, 1993; Nagatani *et al.*, 1993; Reed *et al.*, 1994; Oyama *et al.*, 1997; Guo *et al.*, 1998; Ahmad *et al.*, 1998a). Phenotypic differences of wild-type plants and photoreceptor mutant seedlings (*phyA, phyB, phyA/phyB, cry1, cry2, cry1/cry2, hy5*) grown for seven days under a 16-h photoperiod were compared to those grown for seven days in complete darkness and are presented in Figure 6. While *phyA* and *cry2* mutants exhibited no difference compared to the wild type under light condition, all others showed elongated hypocotyls growth (Figure 6A; Batschauer *et al.*, 2007; Franklin and Quail, 2010). Furthermore, in *cry1/cry2* mutants opening of the hypocotyl hook was slightly delayed. As expected, all dark grown seedlings showed the typical etiolated phenotype (Figure 6B), which is characterized by an elongated hypocotyl, not fully developed cotyledons within an apical hook, and the lack of chlorophyll (Franklin and Quail, 2010).

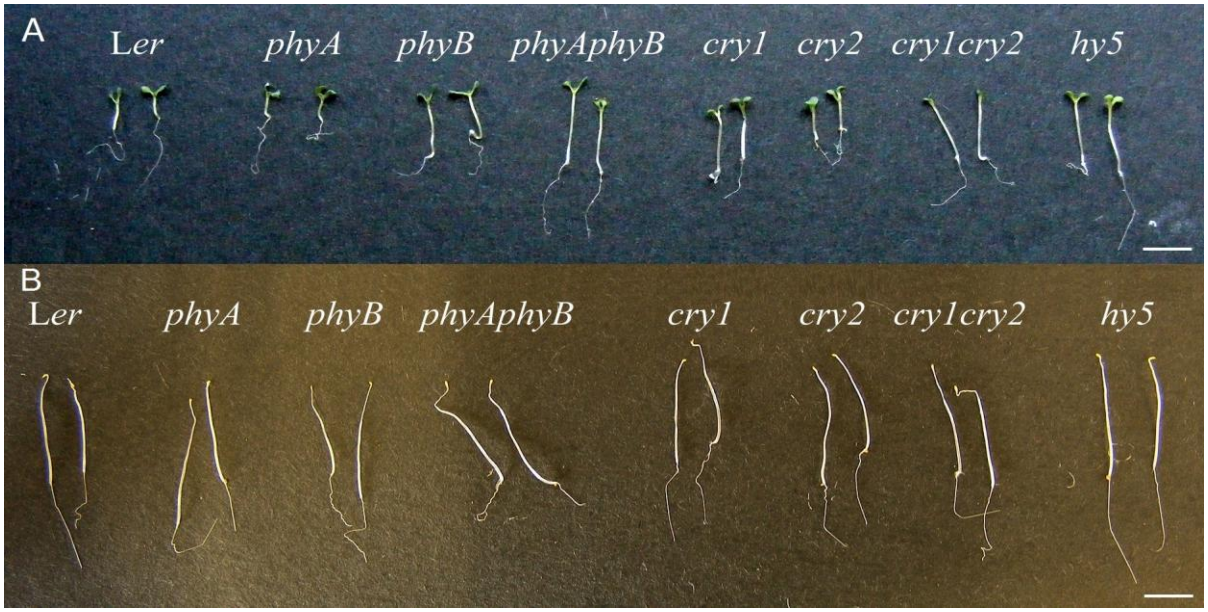

Figure 6: Phenotypic differences of *Arabidopsis* wild type and photoreceptor mutants after seven days in light or darkness.

Seedlings of *Arabidopsis* Landsberg *erecta* (L*er*) wild type and photoreceptor mutants (*phyA, phyB, phyA/phyB, cry1, cry2, cry1/cry2, hy5*) were grown for seven days in white light (A) with a 16-h photoperiod (270 µmol m^{-2} s^{-1}) or in complete darkness (B). Bar = 5 mm.

In addition, phenotypic differences of wild-type and photoreceptor mutant plants grown for seven days in complete darkness and then illuminated for twenty-four hours are presented in Figure 7. Interestingly, red, blue, or green light illumination for twenty-four hours was not sufficient to start a visual de-etiolation of the seedlings. The hypocotyl hook was still closed in all seedlings. This might be due to the short period of illumination or the use of monochromatic light instead of white light. Therefore, the influence of different light qualities on the expression of light-inducible control genes was tested in wild-type plants.

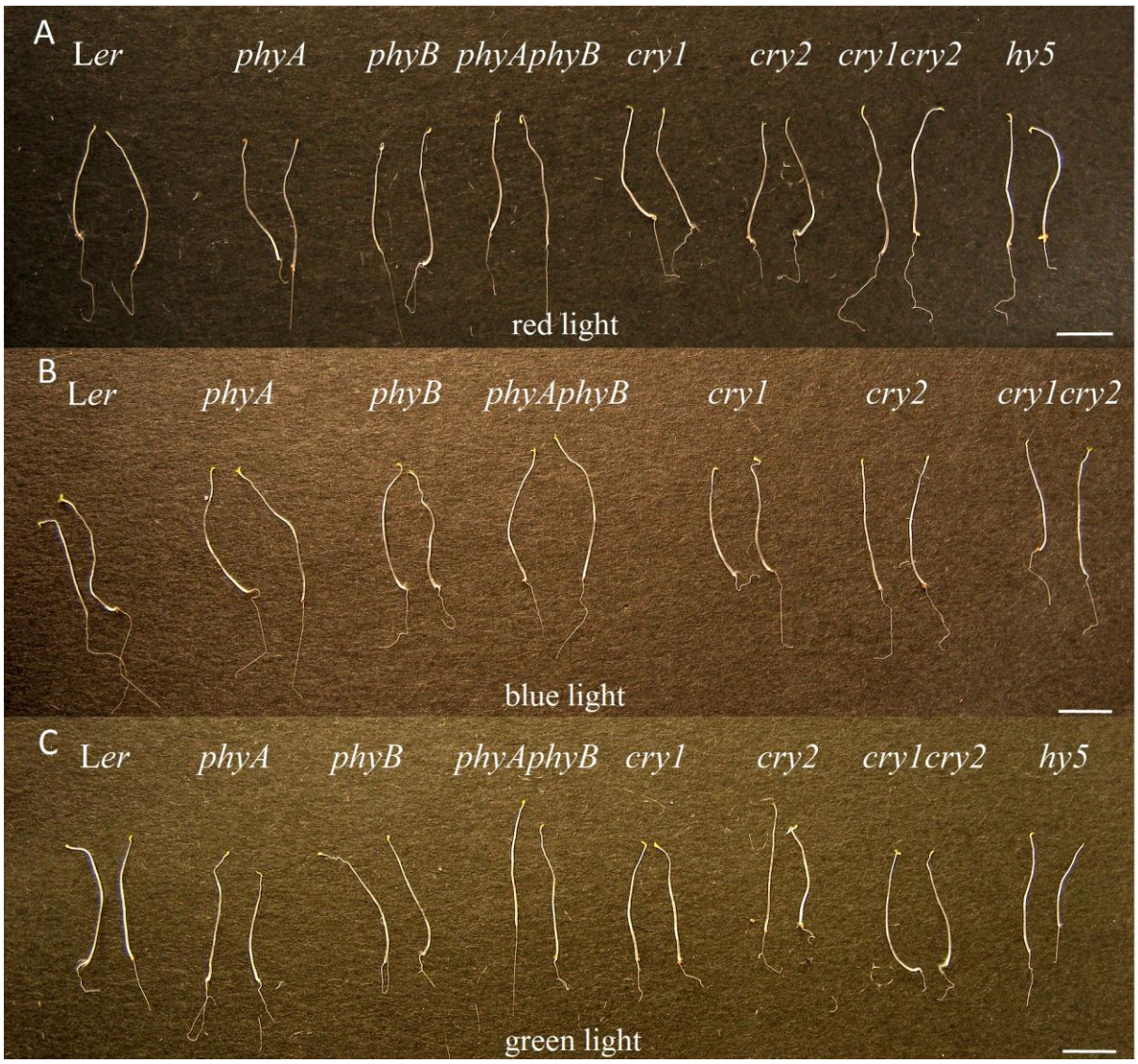

Figure 7: Etiolated wild type and photoreceptor mutants after 24 h exposure to different light conditions.

Seedlings of *Arabidopsis* Landsberg *erecta* (L*er*) wild type and photoreceptor mutants (*phyA, phyB, phyA/phyB, cry1, cry2, cry1/cry2, hy5*) were grown for seven days in complete darkness and then exposed for twenty-four hours to (A) red light (11 µmol m^{-2} s^{-1}), (B) blue light (4 µmol m^{-2} s^{-1}) or (C) green light (3 µmol m^{-2} s^{-1}). Bar = 5 mm.

3.1.1 Expression analysis of light-inducible control genes for L*er* wild type

No phenotypic differences were observed for wild-type plants and photoreceptor mutants after red, blue, and green light illumination. To further examine if the light system used was sufficient to generate clear light signals, the light-regulated expression of three specific light-inducible genes was studied in Landsberg *erecta* (L*er*) wild type (Figure 8). As control for red light inducible changes in the gene expression, the gene encoding auxin-induced protein (*AIP*) was chosen, for blue light the gene encoding chlorophyll A/B binding protein 1 (*cab1*) and for green light the gene encoding early light induced protein1 (*elip1*). Transcript accumulation was analyzed using quantitative real-time PCR with fluorescent TaqMan® probes to allow highly sensitive and specific quantification of gene expression. Light effects on transcript levels were considered significant if the transcript accumulation differed at least 2-fold from the transcript levels in darkness.

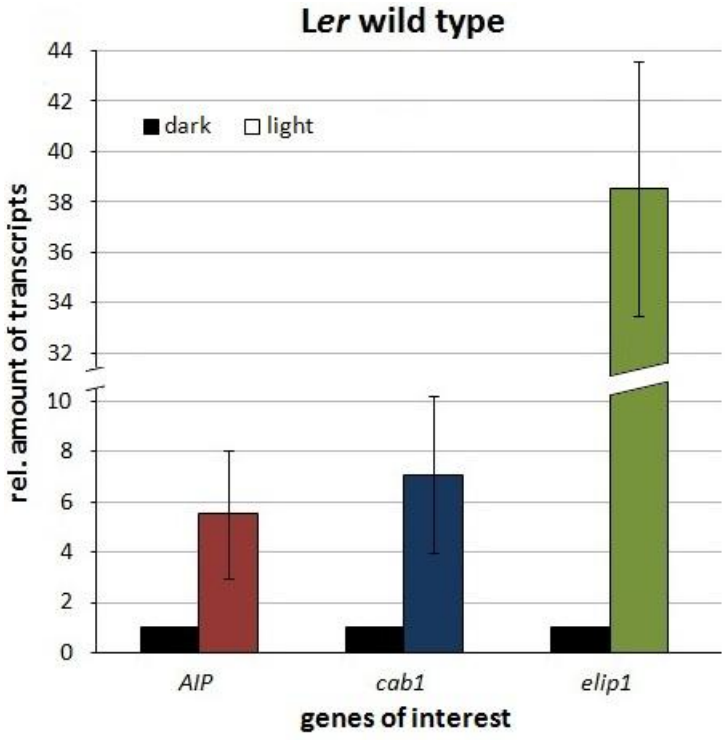

Figure 8: Light-induced changes in the transcript accumulation of control genes.

Seedlings of L*er* wild type were grown in darkness for seven days and subsequently exposed to 6 h red light (11 µmol m^{-2} s^{-1}), 4 h blue light (4 µmol m^{-2} s^{-1}) or 1 h green light (3 µmol m^{-2} s^{-1}), respectively. Samples were taken at the time points indicated. Analysis of transcript accumulation was done by quantitative real-time PCR. Data were normalized to the amounts of transcripts in darkness and are presented as means from two independent experiments ± SE. UBQ11 mRNA levels were used as internal standard. As control for red light inducible changes in the gene expression, the gene encoding auxin-induced protein (*AIP*) was chosen, for blue light the gene encoding chlorophyll A/B binding protein 1 (*cab1*) and for green light the gene encoding early light induced protein1 (*elip1*).

The illumination with the specific light qualities led to an increased transcript accumulation of the light-inducible genes compared to the dark control. Knockout of phytochromes under red light conditions as well as knockout of cryptochromes under blue light conditions led to a drastic reduction of the specific gene transcripts, respectively (data not shown). The observed changes in the transcript accumulation of these control genes reflected the estimated effects of the tested light conditions on gene expression as known from literature, even no phenotypic differences were observed (Gao and Kaufman, 1994; Hamazato *et al.* 1997; Teppermann *et al.*, 2004; Dhingra *et al.*, 2006). Consequently, changes in transcript levels of nuclear and plastidial genes in response to different light conditions were further analyzed with the tested experimental system to gain more information about the influence of light on organellar transcription.

3.1.2 Expression analyses of phage-type RNA polymerase (*RpoT*) genes

3.1.2.1 *RpoT* transcript accumulation in white light for L*er* wild type

The role of light in regulating nuclear and plastid gene expression has been widely studied before, but there is only little information available how light modulates the expression of organellar genes. It has been shown previously that steady-state transcript level of all *RpoT* genes increased when wild-type plants of the Col-0 background were illuminated with white light (Preuten, 2010). To exclude ecotype-related influences, the changes of *RpoTm*, *RpoTmp* and *RpoTp* transcript levels during white light exposure were analyzed in L*er* wild-type plants. This additional analysis was needed since the photoreceptor mutants used in further studies were in a L*er* background and various *Arabidopsis* ecotypes may differ in their response to light. For example, proteomic variations between *Arabidopsis* ecotypes were reported as well as differences in the release of volatile compounds in response to insect attacks (Chevalier *et al.*, 2004; Huang *et al.*, 2010).

To analyze the changes of *RpoTm*, *RpoTmp* and *RpoTp* transcript levels during white light exposure in the *Arabidopsis* L*er* ecotypes, seedlings were grown in darkness for seven days and subsequently exposed to white light with RNA samples taken after one, four, six, and twelve hours of illumination. Quantitative real-time PCR revealed an increase of the *RpoT* transcript amounts after illumination within six hours (Figure 9).

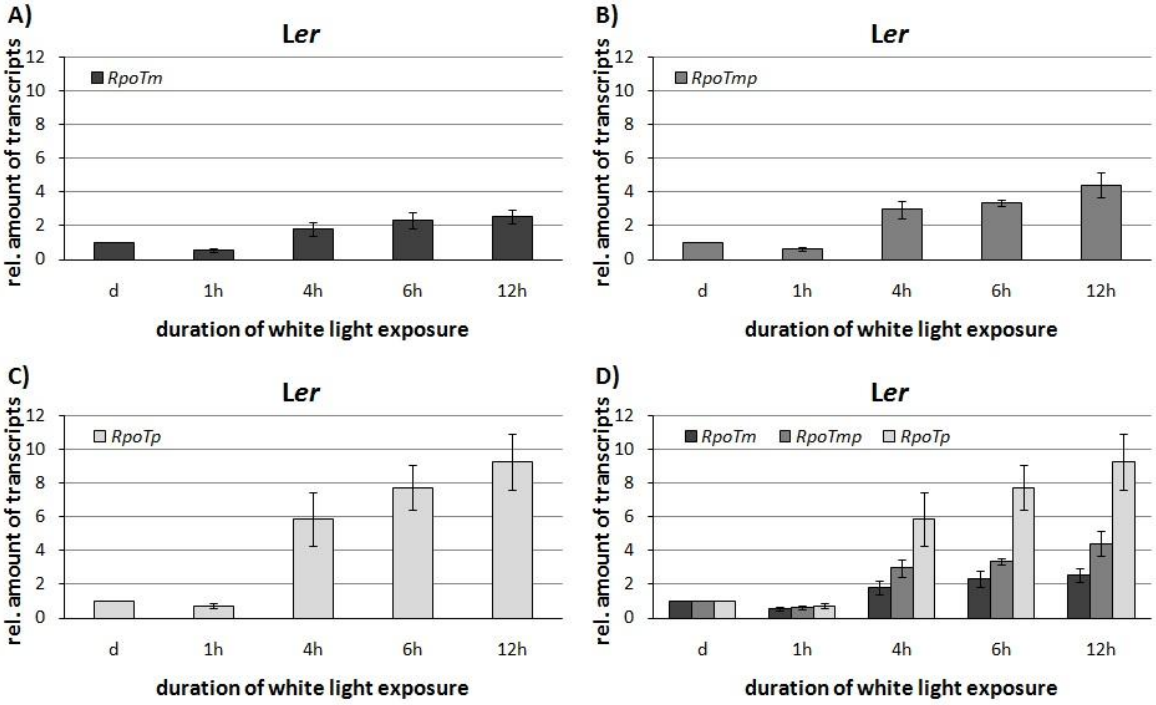

Figure 9: Accumulation of *RpoT* gene transcripts in wild-type plants in white light.

Seedlings of L*er* wild type were grown in darkness (d) for seven days and subsequently exposed to white light (270 µmol m^{-2} s^{-1}). Samples were taken at the time points indicated above. Analysis of *RpoTm* (A), *RpoTmp* (B) and *RpoTp* (C) transcript accumulation was done by quantitative real-time PCR. (D) Synopsis of *RpoT* transcript levels as shown in A-C. Data were normalized to the amounts of *RpoT*s in darkness and are presented as means from two independent experiments ± SE. UBQ11 mRNA levels were used as internal standard.

Brief illumination of up to one hour led to a decrease in the amount of all transcripts to around two thirds of initial levels. Further illumination with white light led to an increase of all transcripts. *RpoTm* transcript levels increased more than twofold of those determined in the dark control after twelve hours (Figure 9A). In contrast, after twelve hours the amount of mRNA for *RpoTmp* was about four times (Figure 9B) and for *RpoTp* more than nine times higher (Figure 9C) than in dark controls. Generally, transcripts for all *RpoT* genes were found to be strongly light induced within six hours (Figure 9D). Light induction was most obvious for *RpoTp*, which encodes the plastid-targeted nuclear-encoded RNA polymerase (Liere *et al.*, 2004). These findings are in accordance to the results of Preuten (2010), where the transcripts of all three *RpoT* genes were found to be strongly light-induced within six hours after illumination. No ecotype-related differences for the transcript accumulation of *RpoT* genes between Col-0 and L*er* wild type were found.

3.1.2.2 *RpoT* transcript accumulation for different light qualities and in mutants

Experiments of Preuten (2010) with different phytochrome and cryptochrome knockout mutants revealed that the influence of different photoreceptors on the accumulation of transcripts of genes encoding the nuclear-encoded organellar phage-type RNA polymerase changes in the course of illumination with white light. To gain more information about the light induced expression of *RpoT* genes and involved pathways, additional analyses of transcript accumulation of *RpoTm*, *RpoTmp* and *RpoTp* in L*er* wild-type plants and different photoreceptor mutants upon illumination with red, blue, and green light using quantitative real-time PCR analyses were performed. To this end, seedlings were grown in darkness for seven days and subsequently exposed to red, blue or green light with RNA samples taken after one, four, six, twelve and twenty-four hours of illumination.

3.1.2.2.1 *RpoT* transcript accumulation in red light for L*er* wild type

In L*er* wild-type plants an increase of all three *RpoT* transcripts was found within six hours of illumination with red light (Figure 10). *RpoTm* and *RpoTmp* transcript levels increased steadily upon exposure to light (Figure 10A+B). Particularly *RpoTp* transcript levels increased quickly (Figure 10C). After twenty-four hours *RpoTp* transcripts were doubled compared to the levels of the transcripts of the two other polymerases (7-fold compared to 3.5-fold). Taken together, *RpoTp* transcript levels increased significantly stronger than those of *RpoTm* and *RpoTmp* (Figure 10D). Red light strongly induces the *RpoT* transcript accumulation in L*er* wild type, indicating that this light-quality might be important for the organellar transcription. To analyze how these light signals are perceived, the influence of red light on *RpoT* transcript levels was further studied in photoreceptor mutants; red light (*phy*) and blue light (*cry*) receptor knockout seedlings; and in knockout mutants for a central signal integrator (*hy5*).

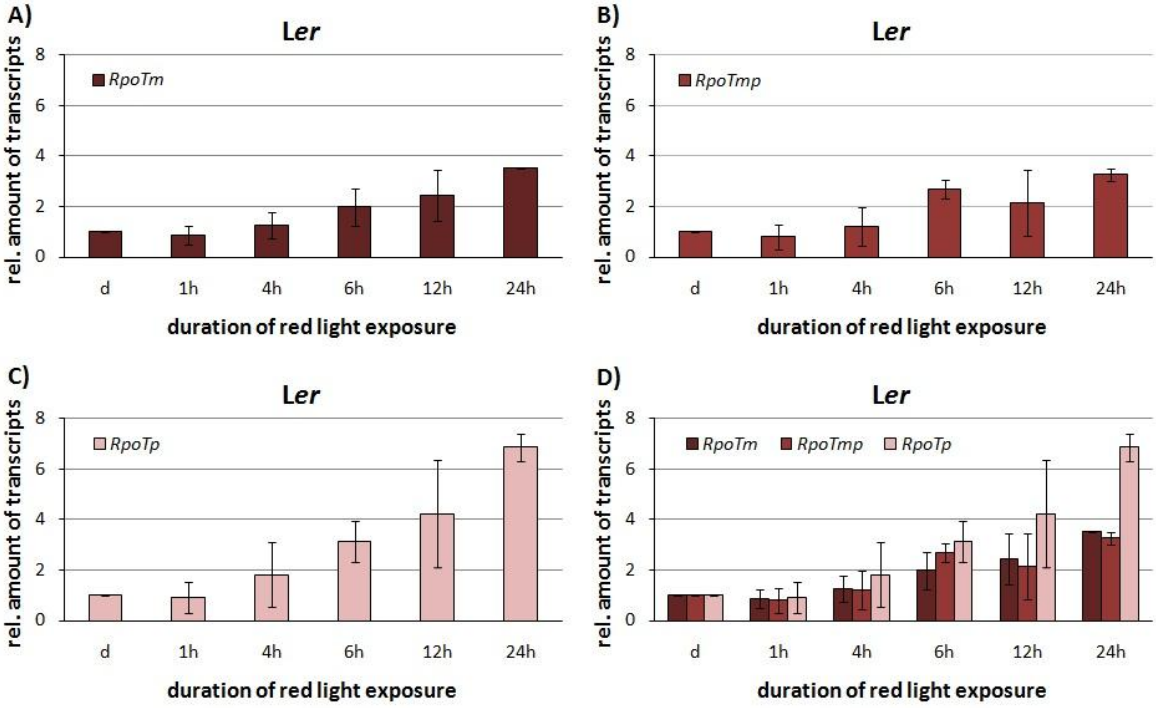

Figure 10: Accumulation of *RpoT* gene transcripts in wild-type plants in red light.

Seedlings of L*er* wild type were grown in darkness (d) for seven days and subsequently exposed to red light (11 µmol m^{-2} s^{-1}). Samples were taken at the time points indicated above. Analysis of *RpoTm* (A), *RpoTmp* (B) and *RpoTp* (C) transcript accumulation was done by quantitative real-time PCR. (D) Synopsis of *RpoT* transcript levels as shown in A-C. Data were normalized to the amounts of *RpoT*s in darkness and are presented as means from two independent experiments ± SE. UBQ11 mRNA levels were used as internal standard.

3.1.2.2.2 *RpoT* transcript accumulation in red light for phytochrome mutants

After one hour of illumination with red light a decrease of *RpoT* transcripts beyond the initial level of dark control was detectable for phytochrome mutants (Figure 11). This effect was most obvious for the *phyB* and *phyA/phyB* mutants, but was not found in wild type. In *phyA* and *phyA/phyB* mutants no induction of *RpoTm* and *RpoTmp* transcripts was found, while in *phyB* mutants a slight induction was detectable. In *phyA* mutants the amount of *RpoTp* transcripts increased slowly upon illumination (Figure 11A). After twenty-four hours *RpoTp* transcript levels were 2.2 times higher than in the dark. In *phyB* mutants the transcript abundance for *RpoTp* increased stronger (Figure 11B). *RpoTp* transcripts accumulated to almost six times higher levels after twelve hours. The accumulation pattern of the *phyA/phyB* mutants resembled that of the *phyB* mutants, but showed a weaker progression (Figure 11C). Apparently, only *RpoTp* gene expression was sufficiently induced by red light in *phy* mutants. However, overall effects were less pronounced than in the wild type (Figure 11D). Therefore, both phytochromes seem to be involved in the perception of red light.

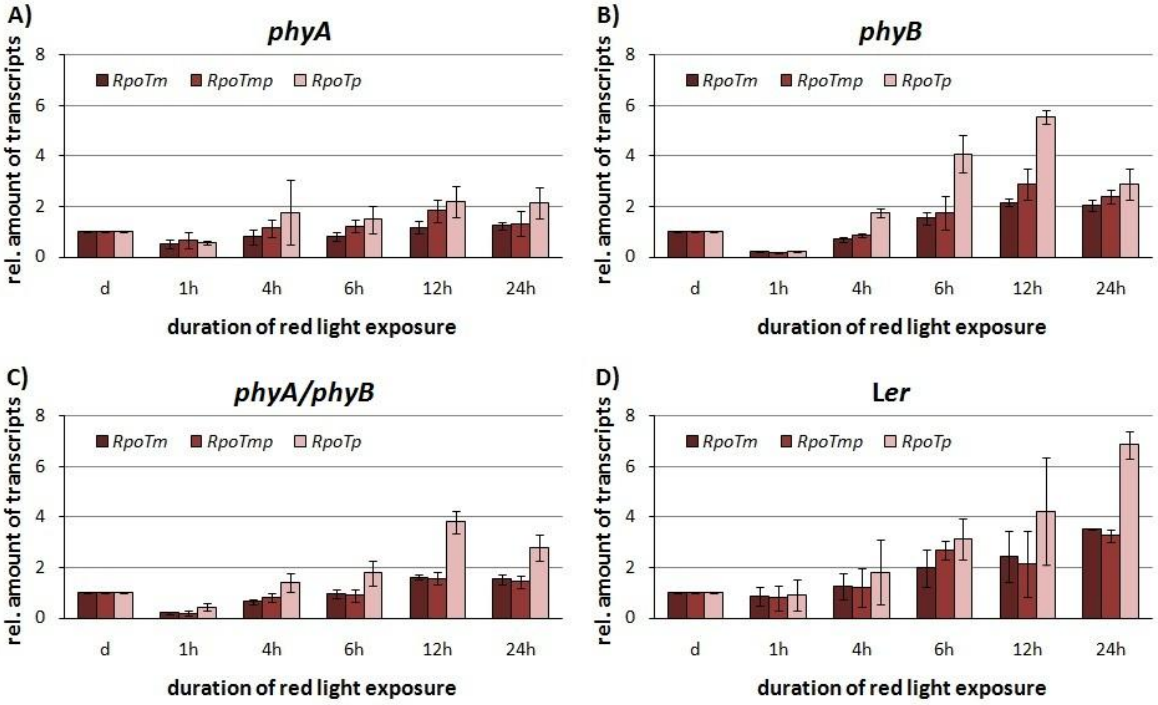

Figure 11: Accumulation of *RpoT* gene transcripts in phytochrome mutants in red light.

Seedlings of *phyA* (A), *phyB* (B) and *phyA/phyB* (C) mutants and L*er* wild type (D) were grown in darkness (d) for seven days subsequently exposed to red light (11 µmol m^{-2} s^{-1}). Samples were taken at the time points indicated above. Analysis of *RpoT* transcript accumulation was done by quantitative real-time PCR. Data were normalized to the amounts of *RpoT*s in darkness and are presented as means from two independent experiments ± SE. UBQ11 mRNA levels were used as internal standard.

3.1.2.2.3 *RpoT* transcript accumulation in red light for cryptochrome mutants

No induction of *RpoTm* and *RpoTmp* transcript levels was observed upon illumination of the blue light receptor knockout mutants *cry1*, *cry2*, and *cry1/cry2* with red light (Figure 12A-C). One exception was seen in *cry1/cry2* mutants were the *RpoTmp* transcript levels at least showed a slight light induction after twelve hours (2-fold increase). In *cry2* mutants, *RpoTp* transcript accumulation peaked after four hours of treatment with red light. Similarly, *RpoTp* mRNA levels in *cry1/cry2* double knockout mutants strongly increased up to four times after twelve hours. However, this induction was not detectable in the single mutants. A knockout of one or both cryptochromes led to a reduction of all *RpoT* transcripts compared to the L*er* wild type (Figure 12D). Comparable transcript levels were found only for *RpoTp* in the *cry1/cry2* mutants after twelve hours of illumination. Nonetheless, the overall transcript accumulation of all three *RpoT*s was strongly inhibited in cryptochrome knockout mutants under red light conditions.

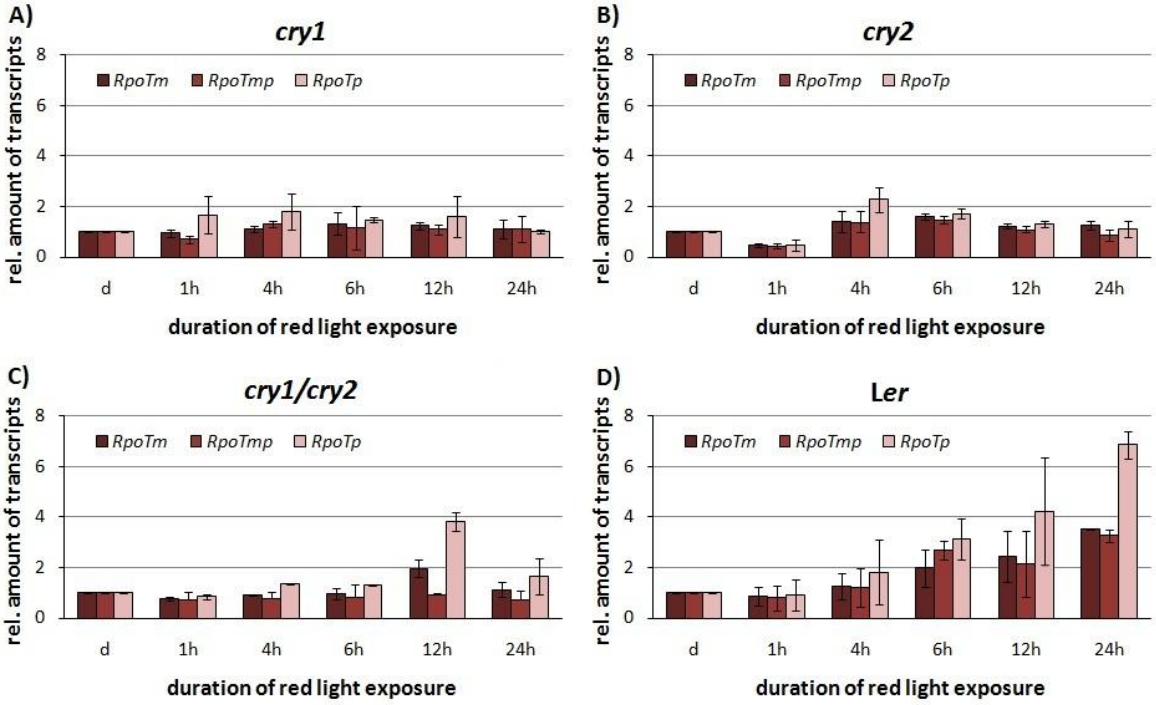

Figure 12: Accumulation of *RpoT* gene transcripts in cryptochrome mutants in red light.

Seedlings of *cry1* (A), *cry2* (B) and *cry1/cry2* (C) mutants and L*er* wild type (D) were grown in darkness (d) for seven days subsequently exposed to red light (11 µmol m^{-2} s^{-1}). Samples were taken at the time points indicated above. Analysis of *RpoT* transcript accumulation was done by quantitative real-time PCR. Data were normalized to the amounts of *RpoT*s in darkness and are presented as means from two independent experiments ± SE. UBQ11 mRNA levels were used as internal standard.

The surprising effect that the blue light receptor knockout seedlings (*cry*) showed no induction of *RpoT* mRNA levels after red light treatment as was seen in wild-type seedlings (Figure 12), raised the question if the appropriate seeds were chosen for the experiments. However, not only the phenotypes of the mutant plants was as expected (Figure 6A), but also Western blot analyses confirmed the mutants to be accurate (Figure 13). Using a PHYA-specific antibody, PHYA was only detected in wild type and *phyB* seedlings (lanes 1 and 3), but not in protein extracts from *phyA* and *phyA/phyB* mutants (lanes 2 and 4). Therefore, the results suggest a role of cryptochromes in mediating red light signals to activate *RpoT* transcript accumulation.

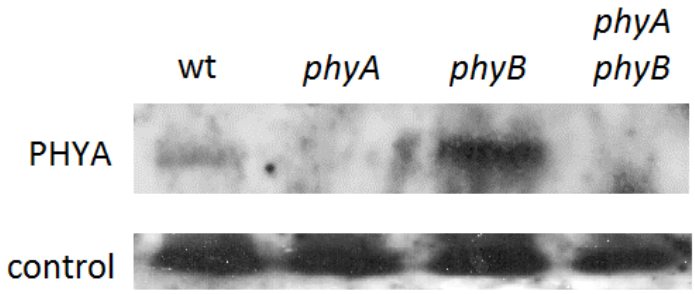

Figure 13: Immunoblot analysis of PHYA in 7-d-old etiolated seedlings of *Arabidopsis* wild type and the phytochrome-deficient mutants.

PHYA was analyzed by immunoblot detection in 7-d-old-etiolated seedlings of *Arabidopsis* L*er* wild type (wt), the phyA mutant (*phyA*), the phyB mutant (*phyB*), and the phyA/phyB double mutant (*phyA /phyB*). For the detection of PHYA a monoclonal antibody was used. Separation of 10 µg total protein on a 7.5% PAA-gel. Control: the large subunit of RuBisCo as detected by Ponceau staining is shown.

3.1.2.2.4 *RpoT* transcript accumulation in red light for *hy5* knockout mutants

The knockout of *hy5* does not completely inhibit light induced accumulation of *RpoTm* and *RpoTp* transcripts, but those of *RpoTmp* (Figure 14A). As seen before in the other mutants, transcript levels of all *RpoT* genes first declined before increasing upon further illumination. Only after twenty-four hours transcripts of *RpoTm* and *RpoTp* accumulated to more than two time higher levels compared to initial values; no increase of *RpoTmp* transcript levels was detectable under red light. The drastic decrease of *RpoT* transcripts in *hy5* mutants suggests HY5 to play an important role in red light transduction to increase *RpoT* transcript levels (Figure 14B).

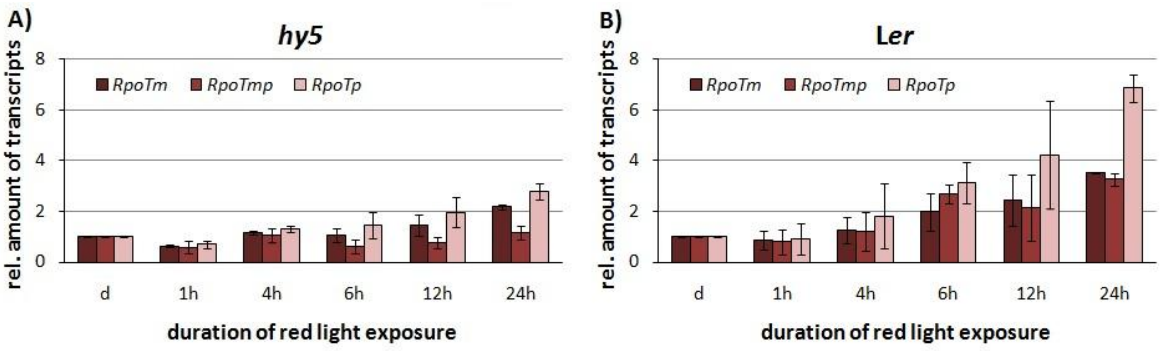

Figure 14: Accumulation of *RpoT* gene transcripts in *hy5* mutants in red light.

Seedlings of *hy5* mutants (A) and L*er* wild type (B) were grown in darkness (d) for seven days subsequently exposed to red light (11 µmol m^{-2} s^{-1}). Samples were taken at the time points indicated above. Analysis of *RpoT* transcript accumulation was done by quantitative real-time PCR. Data were normalized to the amounts of *RpoT*s in darkness and are presented as means from two independent experiments ± SE. UBQ11 mRNA levels were used as internal standard.

3.1.2.2.5 *RpoT* transcript accumulation in blue light for L*er* wild type

Interestingly, blue light was found to have no significant effect on the expression of all three *RpoT* genes in the wild type (Figure 15). A decline of transcripts after one hour was observed. Transcript levels of *RpoTm* (Figure 15A) and *RpoTmp* (Figure 15B) showed a slight increase until twelve hours of illumination and afterwards levels tended to decrease again. *RpoTp* mRNA levels were increased only 1.9 times in maximum (Figure 15C). After one day of illumination the transcript level decreased back to values of the dark control. Taken together, all three RNA polymerase genes showed similar accumulation patterns (Figure 15D) with *RpoTp* transcript levels increasing only slightly stronger than those of *RpoTm* and *RpoTmp*. Overall, however, illumination with blue light did not significantly induce an increase of *RpoT* transcript levels in L*er* wild type.

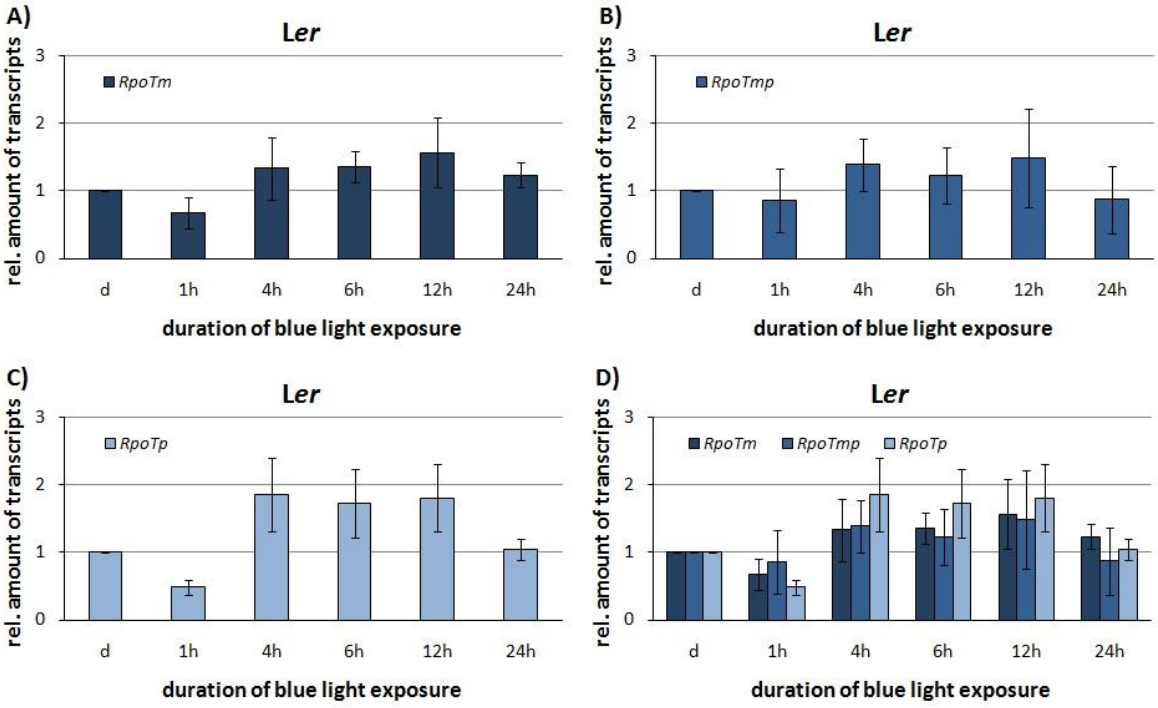

Figure 15: Accumulation of *RpoT* gene transcripts in wild-type plants in blue light.

Seedlings of L*er* wild type were grown in darkness (d) for seven days and subsequently exposed to blue light (4 µmol m^{-2} s^{-1}). Samples were taken at the time points indicated above. Analysis of *RpoTm* (A), *RpoTmp* (B) and *RpoTp* (C) transcript accumulation was done by quantitative real-time PCR. Data were normalized to the amounts of *RpoT*s in darkness and are presented as means from two independent experiments ± SE. (D) Synopsis of *RpoT* transcript levels as shown in A-C. UBQ11 mRNA levels were used as internal standard.

3.1.2.2.6 *RpoT* transcript accumulation in blue light for phytochrome mutants

In *phyA* mutants transcript accumulation of *RpoT* mRNA peaked after twelve hours of light treatment to levels approximately 2- to 4-fold higher than in the dark (Figure 16A). *RpoTp* transcripts were nearly doubled compared to the wild type. In *phyB* mutants, the *RpoT* gene transcript accumulation increased after transfer to blue light (Figure 16B). Again, *RpoTp* transcripts increased most, accumulating to almost three times higher levels after twelve hours in light compared to dark controls. The transcript accumulation in blue light was also studied in *phyA/phyB* double knockout mutants (Figure 16C). In contrast, to the *phy* single knockout mutants, *phyA/phyB* double knockout mutants showed no significant difference to the wild type (Figure 16D). This data indicate that phytochromes are rather not involved in blue light perception.

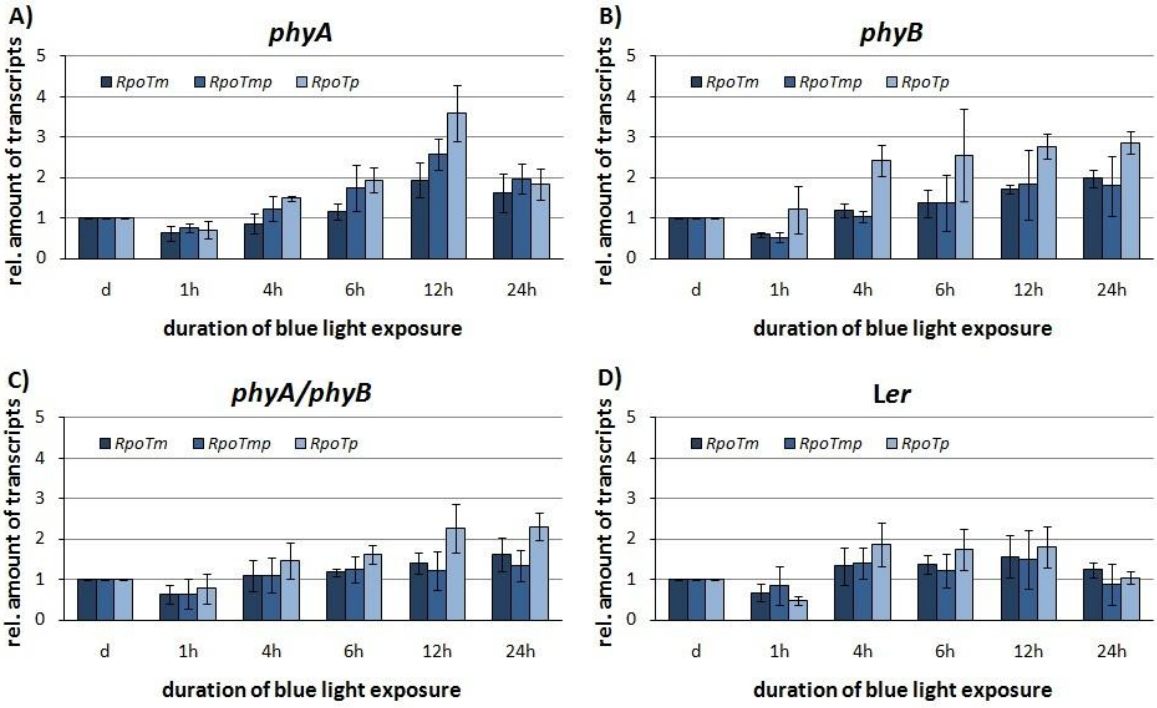

Figure 16: Accumulation of *RpoT* gene transcripts in phytochrome mutants in blue light.

Seedlings of *phyA* (A), *phyB* (B) and *phyA/phyB* (C) mutants and L*er* wild type (D) were grown in darkness (d) for seven days subsequently exposed to blue light (4 µmol m^{-2} s^{-1}). Samples were taken at the time points indicated above. Analysis of *RpoT* transcript accumulation was done by quantitative real-time PCR. Data were normalized to the amounts of *RpoT*s in darkness and are presented as means from two independent experiments ± SE. UBQ11 mRNA levels were used as internal standard.

3.1.2.2.7 *RpoT* transcript accumulation in blue light for cryptochrome mutants

The illumination with blue light did not induce an increase of *RpoTm* and *RpoTmp* transcript levels in the *cry1* and *cry2* mutants (Figure 17A+B). Interestingly, *RpoTp* transcript accumulation was clearly induced by exposure to blue light. The transcript levels were strongly increased within four hours, while longer light exposure did not change the mRNA level significantly further. Surprisingly, the lack of both cryptochromes, CRY1 and CRY2, had an additive effect of blue light on *RpoT* gene expression (Figure 17C). Here, although in case of *RpoTm* and *RpoTmp* only weakly, an influence of light on the transcript accumulation was clearly visible for all three *RpoT* genes, which was not detected in the *cry* single mutants. Within four hours both transcripts reached levels of 2.2 times higher than in dark grown seedlings and stayed constant upon further illumination. *RpoTp* transcript levels increased strongly within four hours of exposure to blue light with a peak value 7.5 times higher than initial values.

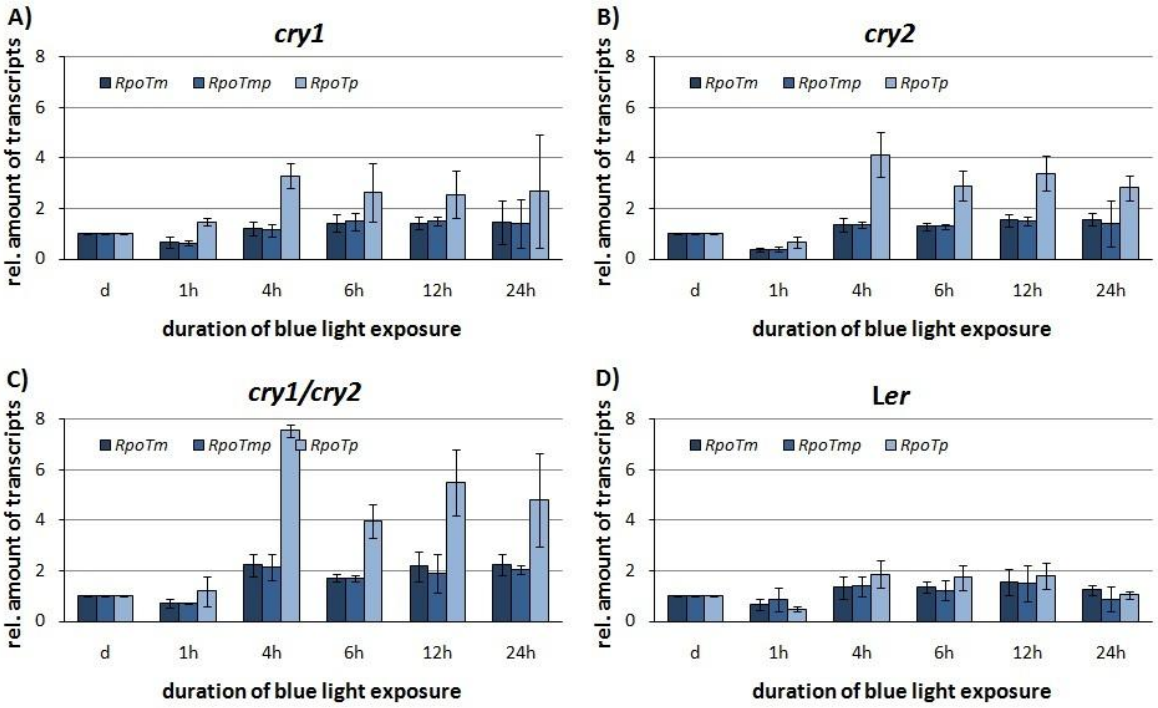

Figure 17: Accumulation of *RpoT* gene transcripts in cryptochrome mutants in blue light.

Seedlings of *cry1* (A), *cry2* (B) and *cry1/cry2* (C) mutants and L*er* wild type (D) were grown in darkness (d) for seven days subsequently exposed to blue light (4 µmol m^{-2} s^{-1}). Samples were taken at the time points indicated above. Analysis of *RpoT* transcript accumulation was done by quantitative real-time PCR. Data were normalized to the amounts of *RpoT*s in darkness. UBQ11 mRNA levels were used as internal standard.

This is in stark contrast to the *RpoT* transcript abundance in the wild type, where blue light did not induce *RpoT* gene expression on transcript levels (Figure 17D). Therefore, the data suggest that indeed cryptochromes mediate blue light signals involved in *RpoT* expression. However, in opposite to red light (Figure 12), blue light signals perceived *via* cryptochromes seem to be rather involved in inhibiting light activation of *RpoT* expression.

3.1.2.2.8 *RpoT* transcript accumulation in blue light for *hy5* knockout mutants

After blue light exposure the *hy5* single knockout mutants showed similar to the *cry* mutants an increase for *RpoTmp* and *RpoTp* transcript levels (Figure 18A). *RpoTmp* transcript accumulation after twenty-four hours showed at least a very weak light induction *via* blue light. Again the strongest effects were detectable for *RpoTp*, where the induction reached a 3.7-fold increase within twenty-four hours of illumination. The *RpoTp* transcript levels in *hy5* mutants were higher than in the wild type (Figure 18B), but not as high compared to the *cry1/cry2* mutants (Figure 17C). Like in the wild type no induction of *RpoTm* transcripts under blue light was detectable. Similar to the cryptochromes, HY5 seems to serve as a central signal integrator involved in mediating blue light signals in repressing *RpoT* expression.

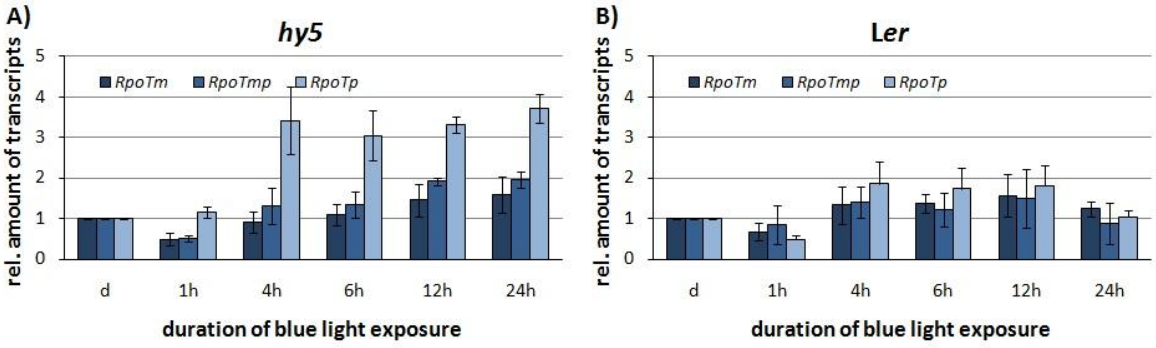

Figure 18: Accumulation of *RpoT* gene transcripts in *hy5* mutants in blue light.

Seedlings of *hy5* mutants (A) and L*er* wild type (B) were grown in darkness (d) for seven days subsequently exposed to blue light (4 µmol m^{-2} s^{-1}). Samples were taken at the time points indicated above. Analysis of *RpoT* transcript accumulation was done by quantitative real-time PCR. Data were normalized to the amounts of *RpoT*s in darkness and are presented as means from two independent experiments ± SE. UBQ11 mRNA levels were used as internal standard.

3.1.2.2.9 *RpoT* transcript accumulation in green light for L*er* wild type

The exposition to green light showed a very interesting accumulation pattern for the wild type (Figure 19A-C). After four hours of illumination an increase of all three *RpoT* transcript amounts was detectable. Although most pronounced for *RpoTp* transcript levels, *RpoTm* and *RpoTmp* mRNAs accumulated also to significant higher levels at this point in time (Figure 19D). Further light exposure led to a decrease of all transcripts back to levels found in darkness. Although often discussed as having less influence on organellar gene transcription when compared to red and blue light, here, green light treatment led to a distinct, albeit brief increase in *RpoT* transcript levels.

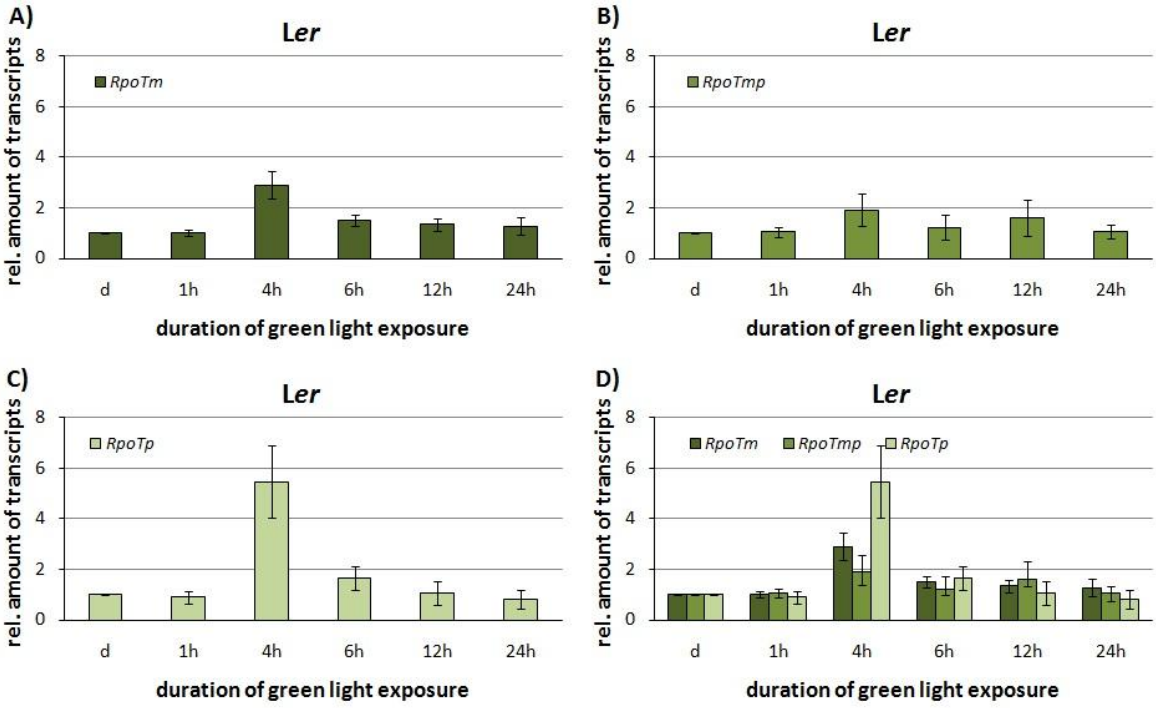

Figure 19: Accumulation of *RpoT* gene transcripts in wild-type plants in green light.

Seedlings of L*er* wild type were grown in darkness (d) for seven days and subsequently exposed to green light (3 µmol m^{-2} s^{-1}). Samples were taken at the time points indicated above. Analysis of *RpoTm* (A), *RpoTmp* (B) and *RpoTp* (C) transcript accumulation was done by quantitative real-time PCR. (D) Synopsis of *RpoT* transcript levels as shown in A-C. Data were normalized to the amounts of *RpoT*s in darkness and are presented as means from two independent experiments ± SE. UBQ11 mRNA levels were used as internal standard.

3.1.2.2.10 *RpoT* transcript accumulation in green light for phytochrome mutants

The *phyA* and *phyB* mutants showed a drop of steady-state transcript levels for all *RpoT*s during four hours of illumination with green light (Figure 20A+B). After six hours, transcript levels started to exceed level of dark control values. Only *RpoTp* transcripts slightly increased beyond the level of two. In the *phyA/phyB* mutants steady-state levels of all *RpoT* genes did not change until twenty-four hours of light treatment (Figure 20C). At that moment, transcript abundance for all three *RpoT*s was just doubled compared to dark controls. Particularly noticeable is the complete lack of the distinct peak after four hours of green light treatment observed in wild-type seedlings (Figure 20D). Therefore, red light absorbing phytochromes seem to be involved in the perception of green light signals.

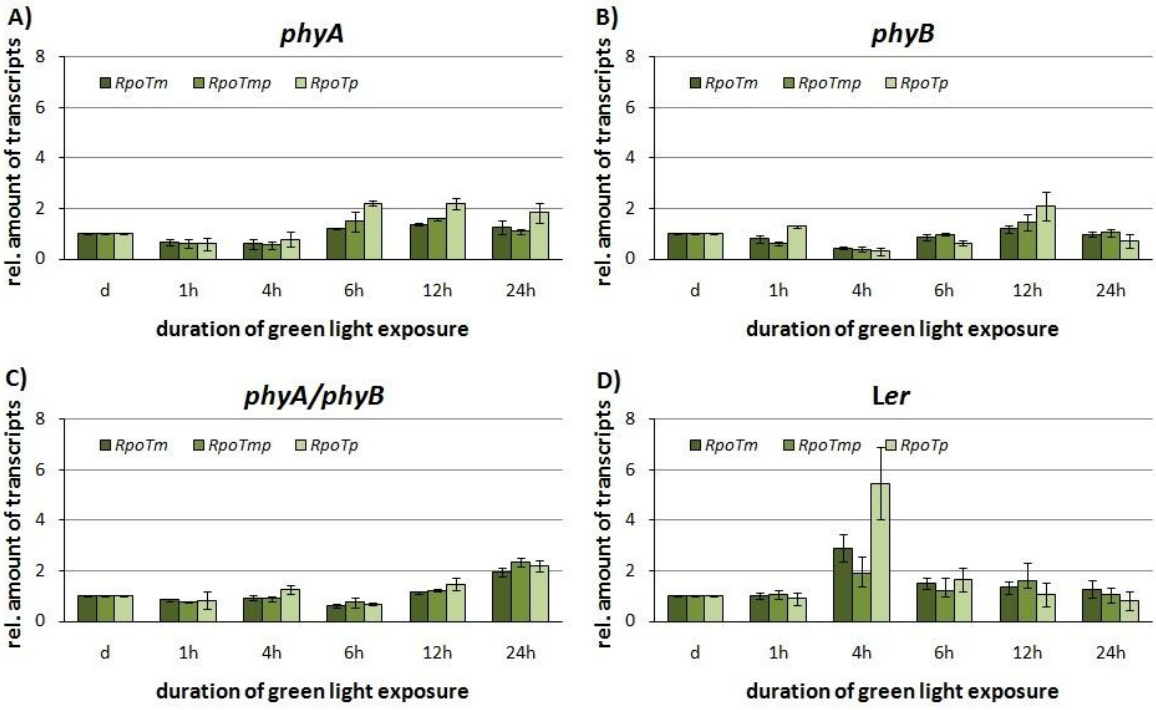

Figure 20: Accumulation of *RpoT* gene transcripts in phytochrome mutants in green light.

Seedlings of *phyA* (A), *phyB* (B) and *phyA/phyB* (C) mutants and L*er* wild type (D) were grown in darkness (d) for seven days subsequently exposed to green light (3 µmol m^{-2} s^{-1}). Samples were taken at the time points indicated above. Analysis of *RpoT* transcript accumulation was done by quantitative real-time PCR. Data were normalized to the amounts of *RpoT*s in darkness and are presented as means from two independent experiments ± SE. UBQ11 mRNA levels were used as internal standard.

3.1.2.2.11 *RpoT* transcript accumulation in green light for cryptochrome mutants

The accumulation of all three *RpoT* genes in the *cry1* mutants was induced by exposure to green light within six hours (Figure 21A). After six hours *RpoTm* and *RpoTmp* transcripts reached levels of around 2 times higher than in dark-grown seedlings. Related to the dark control, mRNA level of *RpoTp* were around 4.8 times in maximum at the same moment and decreased afterwards. In the *cry2* mutants *RpoTm* and *RpoTmp* mRNA accumulation had not been stimulated at all after illumination with green light (Figure 21B). In contrast, after twenty-four hours *RpoTp* transcript levels accumulated to approximately 3.5 times higher levels compared to dark controls. The accumulation pattern for the *cry1/cry2* double mutants resembles that of the *cry2* single mutants (Figure 21C). In the *cry1* single knockout mutants the peak for the *RpoT* genes was just shifted from four hours to six hours compared to the wild type (Figure 21D). The knockout of *cry2* led to strong reduction of *RpoT* transcripts, indicating its major role in green light perception, while the knockout of *cry1* led just to a shift of the transcript accumulation peak.

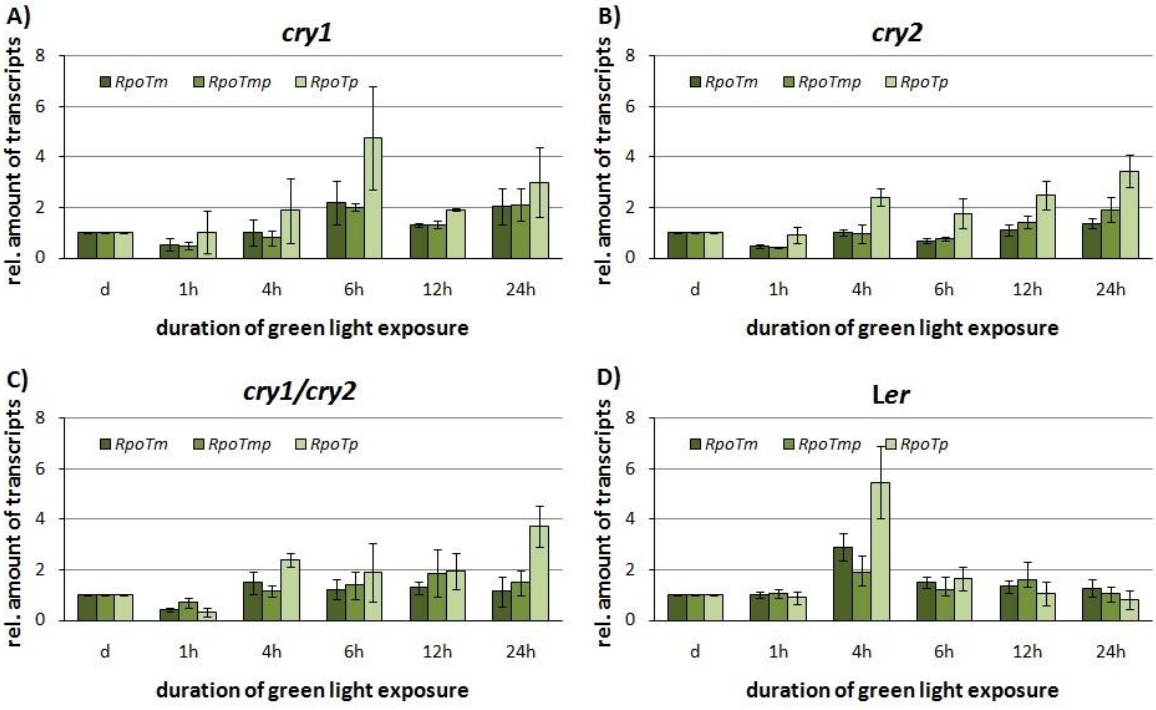

Figure 21: Accumulation of *RpoT* gene transcripts in cryptochrome mutants in green light.

Seedlings of *cry1* (A), *cry2* (B) and *cry1/cry2* (C) mutants and L*er* wild type (D) were grown in darkness (d) for seven days subsequently exposed to green light (3 µmol m^{-2} s^{-1}). Samples were taken at the time points indicated above. Analysis of *RpoT* transcript accumulation was done by quantitative real-time PCR. Data were normalized to the amounts of *RpoT*s in darkness and are presented as means from two independent experiments ± SE. UBQ11 mRNA levels were used as internal standard.

3.1.2.2.12 *RpoT* transcript accumulation in green light for *hy5* knockout mutants

The continuous illumination with green light apparently led to no change for all *RpoT* transcript levels in *hy5* mutants compared to dark control values (Figure 22A). The characteristic peak after four hours of light treatment, which was detectable in the wild type (Figure 22B) was completely missing in the *hy5* mutants (Figure 22A). Generally, detected transcript levels for all three polymerases were found to stay around the level of the dark control, which suggests that HY5 is important not only for red and blue light, but also for green light signal transduction.

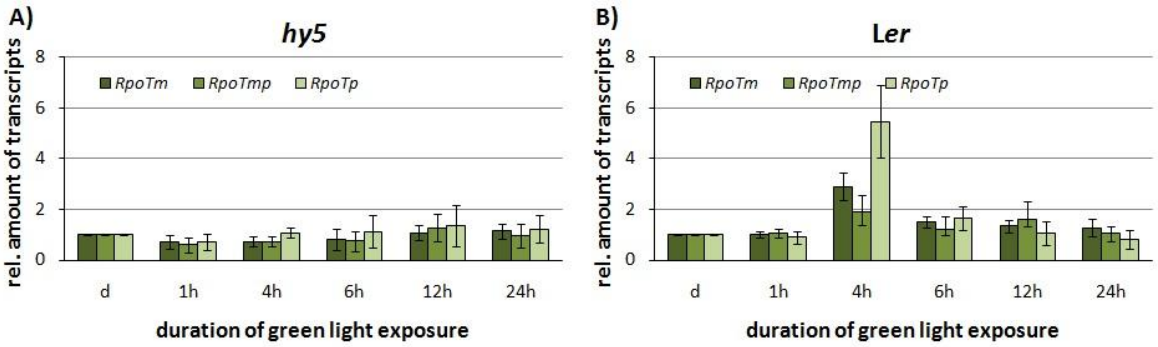

Figure 22: Accumulation of *RpoT* gene transcripts in *hy5* mutants in green light.

Seedlings of *hy5* mutants (A) and L*er* wild type (B) were grown in darkness (d) for seven days subsequently exposed to green light (3 µmol m^{-2} s^{-1}). Samples were taken at the time points indicated above. Analysis of *RpoT* transcript accumulation was done by quantitative real-time PCR. Data were normalized to the amounts of *RpoT*s in darkness and are presented as means from two independent experiments ± SE. UBQ11 mRNA levels were used as internal standard.

3.1.2.3 Summary: *RpoT* transcript accumulation in different light qualities

The observed changes in transcript accumulation indicated that white, red, blue, and green light differentially stimulated the expression of all three *RpoT* genes. In L*er* wild-type plants the illumination with white or red light led to an increase of all *RpoT* transcripts. In contrast, blue light was found to have no significant effect on the expression, while during green light treatment the *RpoT* transcripts peaked after four hours. Studying the influence of red, blue and green light on the *RpoT* gene expression in photoreceptor mutants, a different influence of the receptor classes was revealed. Under red light conditions, phytochromes and cryptochromes seem to be involved in perception of red light signals. The repressive effect of blue light on the *RpoT* transcript accumulation is mediated *via* cryptochromes. Green light signals seemed to be perceived mainly by phytochromes and CRY2, while HY5 was confirmed as an important signal transduction factor under all tested conditions.

3.1.3 Expression analyses of the plastidial *rpoB* gene

To date, the *rpoB* operon, which codes for the beta-subunit of the plastid-encoded plastid RNA polymerase (PEP), is known to be solely transcribed by the nuclear-encoded plastid RNA polymerases in *Arabidopsis* (NEP) (Swiatecka-Hagenbruch *et al.*, 2007). Since both RpoTp and RpoTmp are considered to be NEP (Liere *et al.*, 2011) and therefore responsible for *rpoB* expression, the question arose if an increase in *RpoT* transcript levels translates into higher amounts of *rpoB* RNA. Therefore the effect of light on the transcript accumulation of the plastidial *rpoB* gene was analyzed (Figure 23).

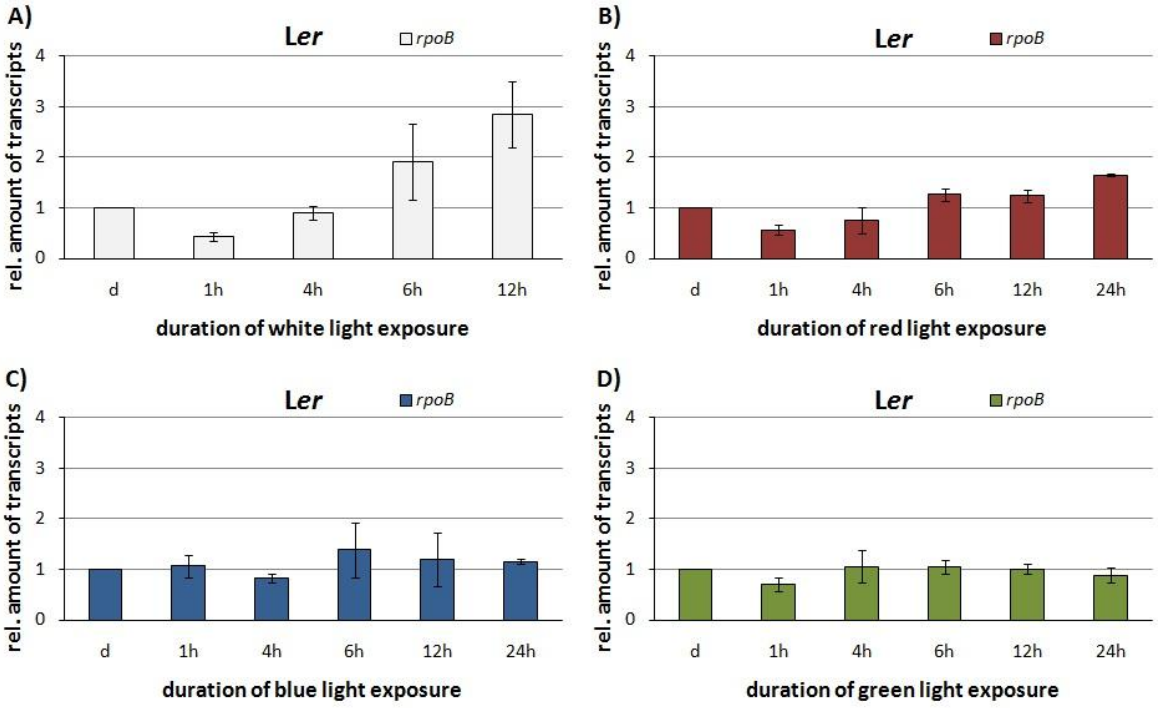

Figure 23: Accumulation of *rpoB* gene transcripts in wild-type plants in different light qualities.

Seedlings of L*er* wild type were grown in darkness (d) for seven days and subsequently exposed to white light (A; 270 µmol m^{-2} s^{-1}), red light (B; 11 µmol m^{-2} s^{-1}), blue light (C; 4 µmol m^{-2} s^{-1}) and green light (D; 3 µmol m^{-2} s^{-1}), respectively. Samples were taken at the time points indicated above. Analysis of *rpoB* transcript accumulation was done by quantitative real-time PCR. Data were normalized to the amounts of *rpoB* in darkness and are presented as means from two independent experiments ± SE. UBQ11 mRNA levels were used as internal standard.

Although showing a distinct decrease after one hour of white light treatment, further illumination showed steadily increasing *rpoB* gene transcript accumulation until twelve hours after transfer to light (Figure 23A). The *rpoB* transcript accumulation occurred about eight hours later compared to the observed increase of *RpoTmp* and *RpoTp* transcripts under the same light conditions (Figure 9B+C) suggesting that enhanced transcript levels of these *RpoT* genes indeed translate into higher NEP activity in plastids.

However, under additional tested blue, red and green light conditions, no induction of *rpoB* gene expression was detectable during twenty-four hours of light treatment (Figure 23B-D). This is in contrast to *RpoTmp* and *RpoTp*, which were light-inducible even by monochromatic light qualities. This suggests that additional factors might be involved in inducing *rpoB* transcript accumulation in response to light, which need more than one monochromatic light quality for full activation.

3.2 Analysis of cytokinin effects on the organellar gene transcription

3.2.1 Influence of cytokinin in cytokinin-related mutants grown on medium

The plant hormone group of cytokinins is known to play an important role during the plant life cycle regulating several important aspects of development. For example, cytokinins possess stimulatory activities in the shoot, while having negative regulatory effects in controlling of root elongation and branching (see for review Schmülling, 2004). The focus of this study is on the role of the histidine kinase receptors AHK2, AHK3, and AHK4/CRE1 as well as of the type-B response regulator ARR1 in the cytokinin-induced chloroplast transcription.

3.2.1.1 Characterization of cytokinin-related mutants

Interested in the potential role of the cytokinin receptors AHK2, AHK3 and AHK4/CRE1 in the transduction of cytokinin signals to the chloroplast, the influence of cytokinin on plastidial transcription in receptor mutants was analyzed. Single knockout receptor mutants (*ahk2*, *ahk3*, *cre1*) as well as double knockout receptor mutants (*cre1/ahk2*, *cre1/ahk3*, *ahk2/ahk3*) were used in the further experiments (Riefler *et al.*, 2006).

Cytokinin-driven B-type response regulators (ARRs) regulate in general the transcription of their target genes. To analyze to which extent the B-type ARRs contribute to cytokinin-regulated organellar transcription, the influence of exogenous cytokinin on transgenic *35S::ARR1-SRDX* (*ARR1*) plants was studied. It was shown that these plants displayed increased resistance and hence reduction of the early transcriptional response to cytokinin (Heyl *et al.*, 2008). To further investigate the role of the endogenous cytokinin pool on the effect of exogenously applied cytokinin on the plastid transcription, cytokinin-deficient transgenic *Arabidopsis* plants were included in the experiments. *35SAth::CKX1* (*CKX1*) plants overexpress the cytokinin oxidase/dehydrogenase 1 gene (AtCKX1). The amount of enzymes which catalyze the degradation of cytokinin is enhanced and hence these mutants possess only 30 to 45% of wild-type cytokinin content (Werner *et al.*, 2003).

To document the influence of cytokinin on the development of *Arabidopsis* plants, photographs of Col-0 wild type and seven different cytokinin-related mutants grown for ten days on medium, supplemented with or without cytokinin (BA, 6-benzyladenin), were taken (Figure 24). Most mutants did not alter significantly in morphological phenotypes when grown on MS plates without BA supplement. The *cre1*, *ahk2* and *ahk3* single mutants as well as the *ARR1* transgenic plants exhibited seedling phenotypes comparable to the wild type. The *ahk* double mutants start to display secondary lateral root branching, which is not seen in wild type. In comparison to wild-type plants, the *ahk2/ahk3* double mutants and the cytokinin-deficient *CKX1* plants interestingly displayed smaller phenotypes and downwards bending of the cotyledons.

Furthermore, when grown on BA-containing medium, the root growth of the single and double cytokinin receptor mutants was as sensitive to BA as the wild-type control. Similarly, the cytokinin-resistance *ARR1* and the cytokinin-deficient *CKX1* mutants, showed also a negative effect of exogenously applied BA on root elongation. In contrast, first leaves of most cytokinin-related mutants seemed to be more developed compared to wild-type plants. Cytokinin inhibits root growth and branching, while promotes stem elongation and cell division. Therefore, the phenotypes of the wild-type and mutant plants were as expected.

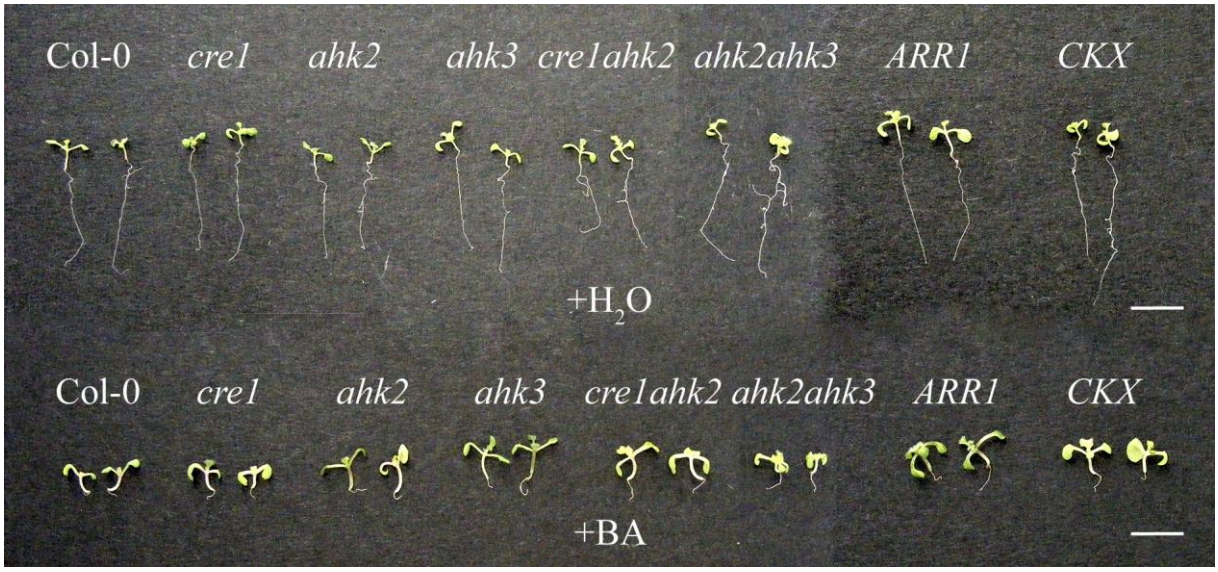

Figure 24: Cytokinin treatment influences mainly the root development.

Wild-type plants (Col-0) and cytokinin-related mutants (*cre1*, *ahk2*, *ahk3*, *cre1/ahk2*, *ahk2/ahk3*, *ARR1* and *CKX1*) were grown for ten days on MS plates with 5 µM 6-benzyladenin (BA) or without (H_2O). *cre1/ahk3* mutants are not shown. Seedlings were grown in a 16-h-photoperiod of white light. Bar = 5 mm.

3.2.1.2 Cytokinin regulation of chloroplast size and chloroplast numbers

The exogenous application of cytokinin (BA) led to a change of chloroplast size and number in wild-type plants (Okazaki *et al.*, 2009). These changes could be a result of cytokinin-induced changes in the chloroplast division machinery. To gain more information about involved pathways, the specific effects of cytokinin on chloroplast size and number was studied by microscopy not only in Col-0 wild-type plants, but also in cytokinin-related mutants (*cre1*, *ahk2*, *ahk3*, *cre1/ahk2*, *cre1/ahk3*, *ahk2/ahk3*, *ARR1*, *CKX1*).

As expected, a reduction of the chloroplast size as a result of cytokinin application was found in Col-0 wild-type plants (Figure 25). The mean chloroplast diameter was 6.5 µm in water-control plants compared to 5.0 µm in cytokinin-treated wild-type plants. Reductive effects of cytokinin on the chloroplast size were also observed for the *ahk2* and *cre1* single mutants as well as for the cytokinin-deficient *CKX1* seedlings. A less pronounced reduction of the chloroplast size was found for the *ahk3* single mutant, the *cre1/ahk3* and *cre1/ahk2* double mutants, and for the *ARR1* seedlings. Interestingly, no significant differences in the chloroplast sizes were detectable in *ahk2/ahk3* double mutants grown on plates with cytokinin to water control plants. Compared to the wild type, the chloroplast size was already reduced in *ahk2/ahk3* mutants without any cytokinin treatment.

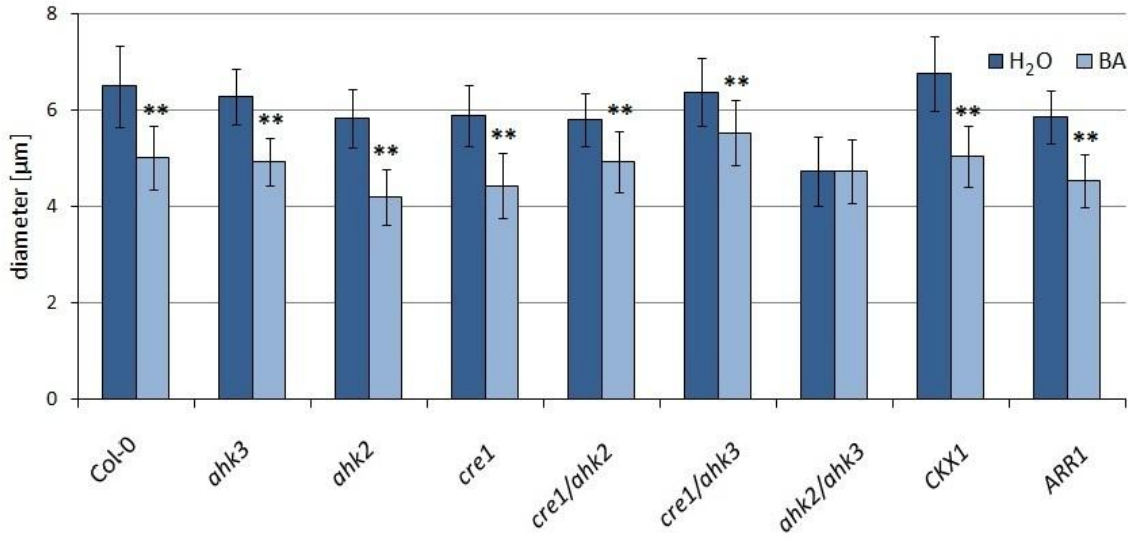

Figure 25: Cytokinin treatment decreases the size of chloroplasts.

Arabidopsis wild type and cytokinin-related mutant seedlings were grown for ten days on MS plates with 5 µM 6-benzyladenin (BA) or without (H_2O). Cytokinin effects on chloroplast size are presented. For determination of chloroplast size means ± SE from each hundred individual chloroplasts are shown. Asterisks indicate a significant difference as calculated by Student's *t* test ($p < 0.005$).

Taken together, exogenously applied cytokinin appears to restrict chloroplast size as the diameter of chloroplasts in cytokinin-treated plants was smaller than in non-treated plants. The knockout of the two histidine kinases *ahk2* and *ahk3* led to complete inhibition of cytokinin-induced chloroplast size reduction. It is known, that both cytokinin receptors contribute to the cytokinin-regulated leaf cell formation and therefore the formation of chloroplasts might also be negatively affected in the double receptor mutants (Riefler *et al.*, 2006).

Additionally, the average number of chloroplasts in mesophyll cells was determined for plants grown with or without application of cytokinin (Figure 26). This was done to analyze if the chloroplast size changed to compensate for altered numbers of chloroplasts per cell. For wild-type seedlings an about 1.5-fold higher number of chloroplasts was found in treated plants compared to the water control. For comparison, the largest difference between plants grown on medium with or without cytokinin was seen for *ARR1* plants. The chloroplast numbers increased significantly in nearly all cytokinin-treated mutants compared to those in plants grown without cytokinin, but to different extent. Only in cytokinin-deficient *CKX1* seedlings treatment with BA did not increase the number of chloroplasts.

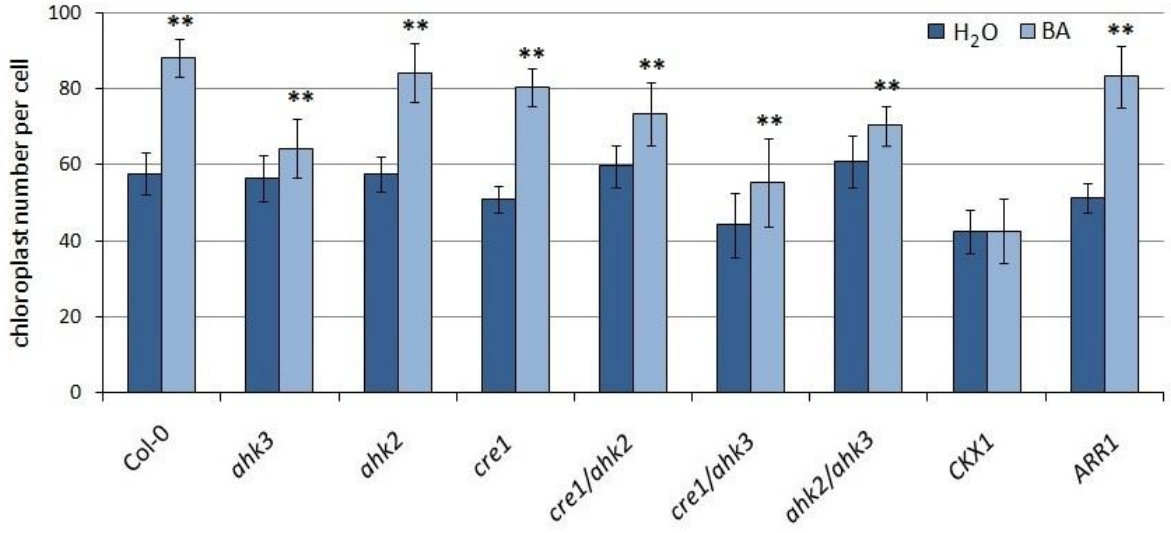

Figure 26: Cytokinin treatment increases the number of chloroplasts per mesophyll cell.

Arabidopsis wild type and cytokinin-related mutant seedlings were grown for ten days on MS plates with 5 µM 6-benzyladenin (BA) or without (H_2O). Cytokinin effects on chloroplast numbers are presented. For determination of chloroplast numbers means ± SE from analyzing each 17 mesophyll cells are shown. Asterisks indicate a significant difference as calculated by Student's *t* test ($p < 0.005$).

To summarize, with exception of the cytokinin-deficient *CKX1* (no change in chloroplast number) and *ahk2/ahk3* mutants (no change in chloroplast size), exogenously applied cytokinin led to a larger number of smaller chloroplasts in most of the analyzed *Arabidopsis* plants. The results suggest that cytokinin led to an increase in the chloroplast division rate and hence to alterations of chloroplast parameters such as size and number. The presence of smaller chloroplast might also influence the plastome copy numbers per cell, therefore this plastidial parameter was studied next.

3.2.1.3 Cytokinin effects on the plastome copy numbers per cell

Endoreduplication is a common mechanism in plants, which leads to changes in nuclear DNA content. It is known from previous experiments in *Arabidopsis* that the mean ploidy levels (*C*-values, where 2C equals diploidy) can vary due to endoreduplication events (Barow and Meister, 2003; Zoschke *et al.*, 2007). Furthermore, knowledge of nuclear ploidy levels is a prerequisite to quantify plastidial gene copy numbers relative to the nuclear DNA content. To study, if the increasing number of smaller chloroplasts per cell in cytokinin-treated plants has an influence on nuclear DNA ploidy levels (Figure 27) and plastome copy numbers (Figure 28), additional flow-cytometric analysis was performed.

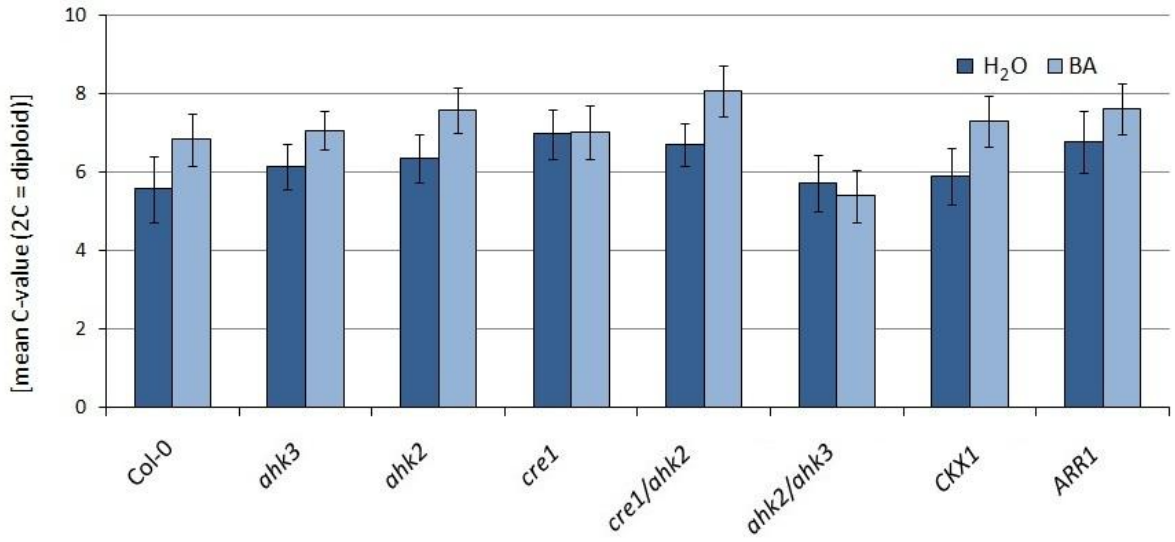

Figure 27: Cytokinin effects on genome ploidy levels in Col-0 wild type and mutant seedlings.

Arabidopsis wild type and cytokinin-related mutant seedlings were grown for ten days on MS plates with 5 µM 6-benzyladenin (BA) or without (H_2O). Changes in nuclear DNA content (ploidy levels) in different plants was measured by flow cytometry as outlined in Material and Methods section. Means from three independent experiments ± SE are presented.

Interestingly, average ploidy numbers increased slightly in nearly all plants after application of cytokinin by 1C (Figure 27). For example, in Col-0 wild type the average ploidy numbers changed from about 6 to about 7 genome copies per cell. In the cytokinin-related mutants mean *C*-values varied between 6C to 7C for water control conditions and between 7C to 8C for cytokinin treatment. No influence of cytokinin treatment on the nuclear ploidy levels were found in the *cre1* single and the *ahk2/ahk3* double mutants suggesting that the reception of cytokinin is indeed responsible for the observed increase in nuclear ploidy levels.

Using the mean *C*-values (Figure 27) and the quantitative RT-PCR data on the amount of the plastidial genes *psbA* and *clpP*, determination of the average plastome copy numbers per cell was carried out. In general, BA treatment also resulted in an increase of the plastome copy numbers per cell (Figure 28). About 1200 plastome copies per cell were observed in non-treated wild-type plants. This correlates well with estimates of Zoschke *et al.* (2007) where 1000-1700 plastome copies per cell during *Arabidopsis* leaf development were found.

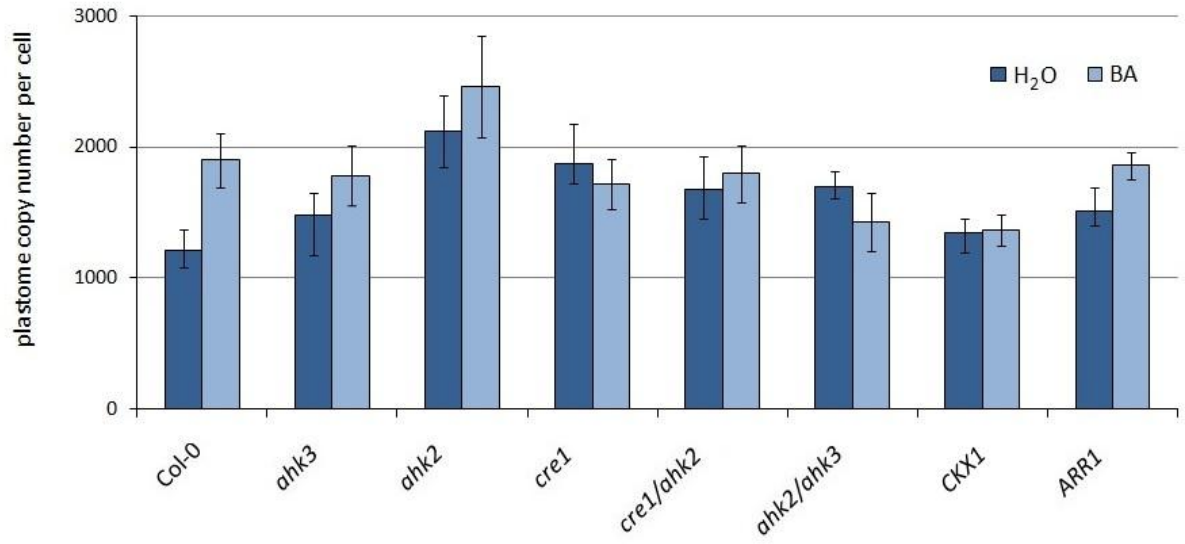

Figure 28: Cytokinin effects on plastome copy numbers in Col-0 wild type and mutant seedlings.

Arabidopsis wild type and cytokinin-related mutant seedlings were grown for ten days on MS plates with 5 µM 6-benzyladenin (BA) or without (H_2O). Plastome copy numbers per cell in different plants are presented. Analysis of relative DNA content of *Arabidopsis* plastidial *psbA* and *clpP* genes in different plants was performed by quantitative real-time PCR. Data were normalized to the amount of nuclear *RpoTm* gene and expressed as $2^{-\Delta C_T}$. Therefore plastome copy numbers per cell were determined by calculating the product of the average values of the two quantified genes and the mean *C*-value. Means from three independent experiments ± SE are presented.

However, in wild-type plants grown on medium with BA a significant rise in copy numbers up to 1900 plastome copies per cell was observed. In *ahk2*, *ahk3*, and *ARR1* seedlings also enhanced copy numbers were detected due to the cytokinin supplementation. Surprisingly, the highest overall copy numbers were found in *ahk2* seedlings both untreated (about 2110 plastome copies per cell) and treated (about 2460 plastome copies per cell). Furthermore, the cytokinin receptor single (*cre1*) and double mutants (*ahk2/ahk3*, *cre1/ahk2*) showed no significant change in their plastome copy numbers due to BA treatment suggesting that cytokinin reception is indeed the reason for increasing plastome copy numbers. As expected, in plants with low endogenous cytokinin levels (*CKX1*) the addition of exogenous BA did not alter the plastome copy numbers.

3.2.1.4 Cytokinin effects on plastid gene transcription

To study if BA treatment and/or the increased plastome copies have an effect on plastid transcription, run-on analyses were performed with chloroplasts isolated from *Arabidopsis* seedlings grown for ten days on MS plates with or without 5 µM BA. The labeled transcripts were hybridized to DNA fragments of 31 chloroplast genes spotted on a nylon membrane. The selected chloroplast genes represented functionally important groups of chloroplast proteins and RNAs as well as NEP- and PEP-transcribed plastidial genes. The studied genes were split in two functional related groups for later analyses, housekeeping genes and photosynthesis-related genes (Table 8). To further investigate if cytokinin signals involved in regulation of chloroplast gene transcription are recognized *via* their standard pathways (To and Kieber, 2008), the transcriptional activity of plastidial genes was not only analyzed in Col-0 wild-type plants, but also in mutants for factors which act either at the level of cytokinin detection (*ahk2/ahk3*) or at the level of the cytokinin response regulation (*ARR1*).

Table 8: Functional groups of chloroplast genes analyzed in run-on assays.

functional group	genes
housekeeping genes	*accD*, *clpP*, *rpoB*, *rps4*, *rps8*, *rps14*, *rps16*, *rrn16*, *trnK1*, *trnK1*, *trnL*, *ycf1*, *ycf2*, *ycf5*, *ycf10*
photosynthesis-related genes	*atpB*, *atpF*, *atpH*, *ndhB*, *ndhI*, *petA*, *petB*, *petD*, *psaA*, *psaB*, *psaC*, *psbA*, *psbD*, *psbE*, *psbK*, *rbcL*

In the wild-type plants and both mutants, the transcription in chloroplasts did not respond to cytokinin (Figure 29). Since the cytokinin effect on chloroplast transcription was considered significant if the run-on signals differed at least 2-fold from the water control, no significant response to BA was found. In all tested plants only an around 1.3-fold higher activation of transcription could be observed as a result of a cytokinin effect. Even for both classes of functional genes there was no significant response to the cytokinin treatment detectable. Therefore, although showing higher plastome copy numbers, the transcription activity in plants grown on medium with cytokinin most likely adapted to the permanent high cytokinin levels.

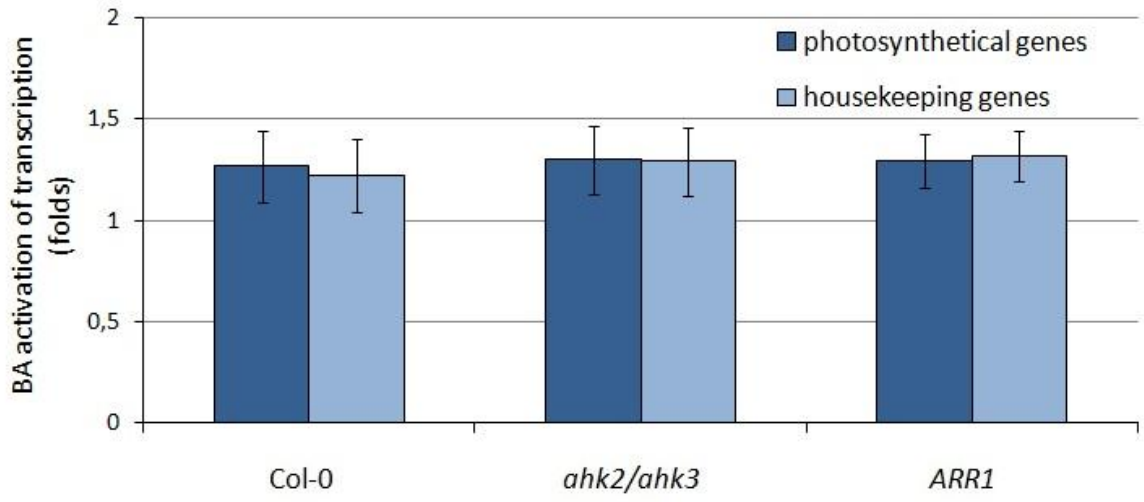

Figure 29: Cytokinin effects on plastidial gene transcription in seedlings sown on MS medium.

Arabidopsis wild-type plants and cytokinin-related mutant seedlings were grown for ten days on MS plates with 5 µM 6-benzyladenin (BA) or without (H_2O). Ratios of the transcription rates in chloroplasts (photosynthesis-related and housekeeping genes) from cytokinin-treated *Arabidopsis* wild type and selected cytokinin-related mutant seedlings to the rates in chloroplasts from non-treated plants are presented as means from at least two independent experiments ± SE.

3.2.2 Cytokinin effects on plastid gene transcription in seedlings sown on a net

To circumvent adaption of plastid transcription to higher cytokinin levels, *Arabidopsis* wild-type plants and cytokinin-related mutants were cultivated on top of a net placed on soil for twelve days and cytokinin treatment was only performed for the last six hours. In experiments with barley, pre-incubation of the detached leaves for twenty-four hours on water in the light before BA application was found to be a prerequisite for pronounced cytokinin effects on chloroplast transcription (Zubo *et al.*, 2008). Therefore, 12-day-old *Arabidopsis*

seedlings were cut, washed and then pre-incubated in water for twenty-four hours under continuous light. Afterwards the plants were transferred for additional six hours to a BA solution (2.2 x 10^{-5}M) or water (control). Again, chloroplast isolation and run-on transcription assay were performed as described.

Chloroplast gene transcription in Col-0 wild-type plants showed a significant response to cytokinin incubation for six hours (Figure 30). The incubation with cytokinin led for photosynthesis-related and housekeeping genes to around 2.3- to 2.4-fold higher activation of transcription, respectively. In contrast, cytokinin treatment of cytokinin-related mutants resulted in no or only slight induction of plastidial gene transcription. The effects of cytokinin on chloroplast transcription were reduced in all three kinase mutants, although more prominent in *ahk3* and *cre1* than in *ahk2*. As expected, the cytokinin effect on plastid transcription was even stronger reduced in *ahk* double mutants than in the single mutants. *ARR1* mutants possess an increased resistance to BA due to the knockout of the response regulator ARR1, while in *CKX1* mutants the cytokinin degradation is enhanced. Consequently, no BA-induced activation of chloroplast gene transcription was observed in cytokinin-deficient *CKX1* and cytokinin-resistant *ARR1* plants.

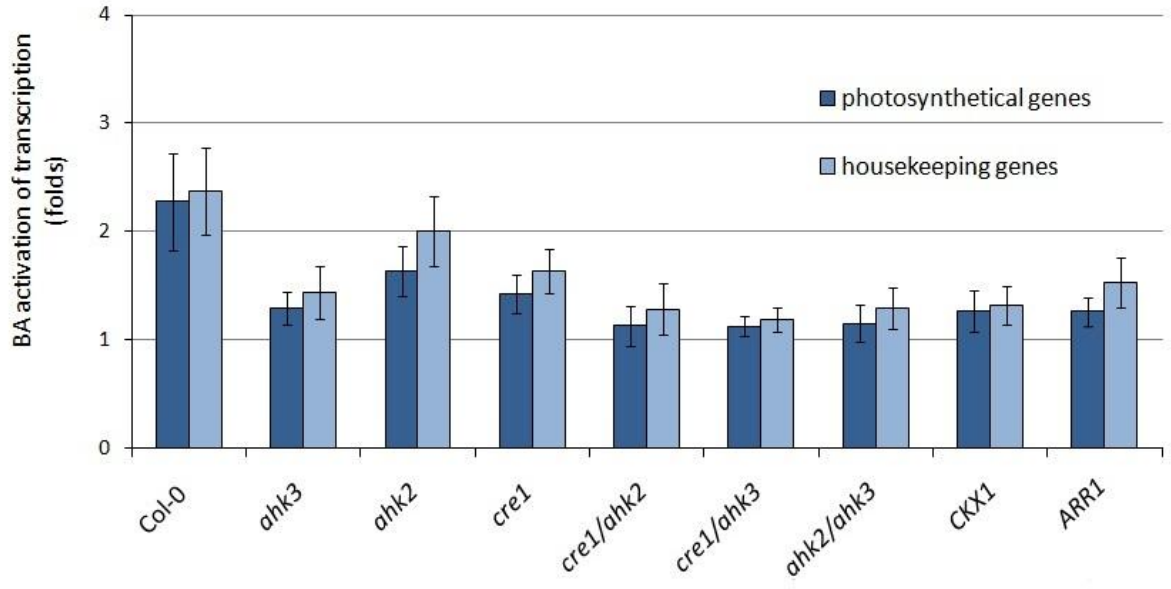

Figure 30: Cytokinin regulation of chloroplast gene transcription in seedlings sown on net.

Ratios of the transcription rates in chloroplasts (photosynthesis-related and housekeeping genes) from cytokinin-treated *Arabidopsis* wild type and cytokinin-related mutant seedlings to the rates in chloroplasts from non-treated plants are presented as means from at least two independent experiments ± SE. Plants were cultivated on top of a net placed on soil. A 24-h pre-incubation of 12-day-old plants on water and subsequent incubation for six hours on water or BA solution was performed. Pre-incubation and incubation of the seedlings were carried out in the light.

The results indicated that histidine kinase receptors are functionally redundant. Nevertheless, influence of mutations in *cre1* and *ahk3* genes stronger abolished cytokinin action on chloroplast transcription then mutation in *ahk2* gene. In addition, different groups of genes were regulated in different extends in receptor mutants (BA activation of transcription of housekeeping genes slightly stronger than those of photosynthesis-related genes).

3.2.3 Cytokinin effects on plastid gene transcription/transcripts in *sig*-mutants

Some of cytokinin-inducible genes were transcribed *via* plastid-encoded plastid RNA polymerase (PEP). To investigate the role of sigma factors needed for promoter recognition by PEP in the cytokinin signaling pathway, the influence of cytokinin on chloroplast transcription was analyzed in sigma factor mutants (*sig*-mutants) by run-on assays (Figure 31) and quantitative real-time PCR (Figure 32).

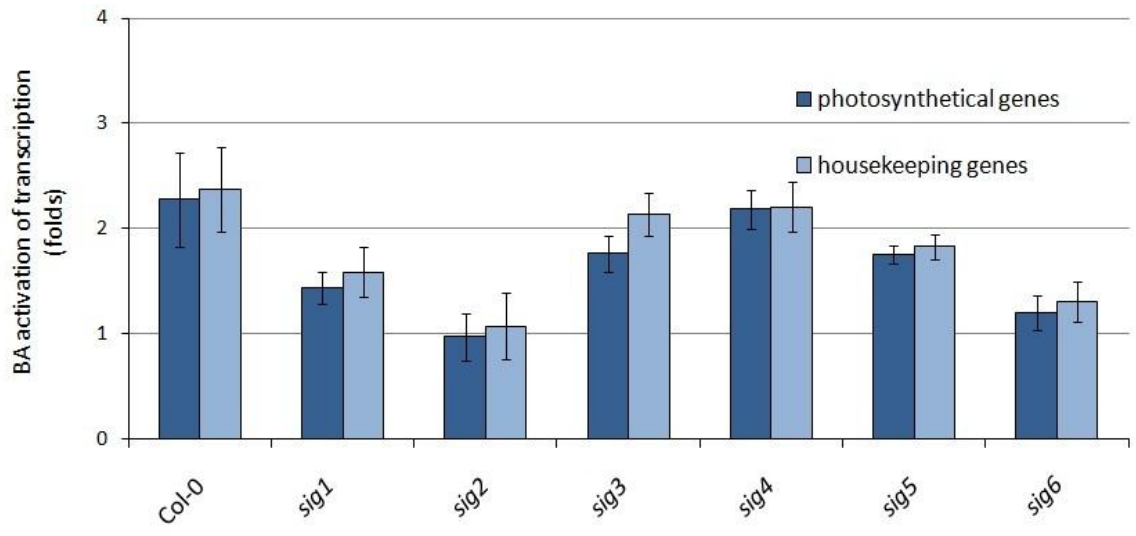

Figure 31: Cytokinin regulation of chloroplast gene transcription in sigma factor mutants.

Ratios of the transcription rates in chloroplasts (photosynthesis-related and housekeeping genes) from cytokinin-treated wild type and sigma factor mutant seedlings to the rates in chloroplasts from non-treated plants are presented as means from at least three independent experiments ± SE. Plants were cultivated on top of a net placed on soil. A 24-h pre-incubation of 12-day-old plants on water and subsequent incubation for 6h on water or BA solution was performed.

An induction of chloroplast gene transcription was found in wild-type plants (Figure 31). Application of exogenous cytokinin led to a more than 2-fold activation of plastidial gene transcription. Similar to cytokinin-related mutants, transcription of housekeeping genes in *sig*-mutants were found to be less affected compared to photosynthesis-related genes. Cytokinin

effects on total chloroplast transcription were slightly reduced in *sig3*, *sig4,* and *sig5* mutants and significantly reduced in *sig1, sig2,* and *sig6* mutants. No induction of plastidial gene transcription *via* BA was found for *sig2* mutants, where the transcription level was less than half of the level in wild type. In contrast, the level of gene transcription in *sig4* mutants was very similar to that in Col-0 seedlings.

The negative effects on BA-induced gene transcription were more pronounced in *sig2* and *sig6* mutants than in the other mutants indicating that the sigma factors SIG2 and SIG6 may be predominantly involved in cytokinin-induced transcriptional changes. However, no effect of cytokinin on the steady-state levels of transcripts of the six sigma factor genes was found in *Arabidopsis* wild-type seedlings (Figure 32). For all tested sigma factor genes, the transcript levels after cytokinin application were as high as the levels under control conditions suggesting activation of PEP transcription in response to cytokinin treatment rather by post-translational modifications of the sigma factors (Pfannschmidt and Liere, 2005) than activation of the expression of the sigma factors themselves.

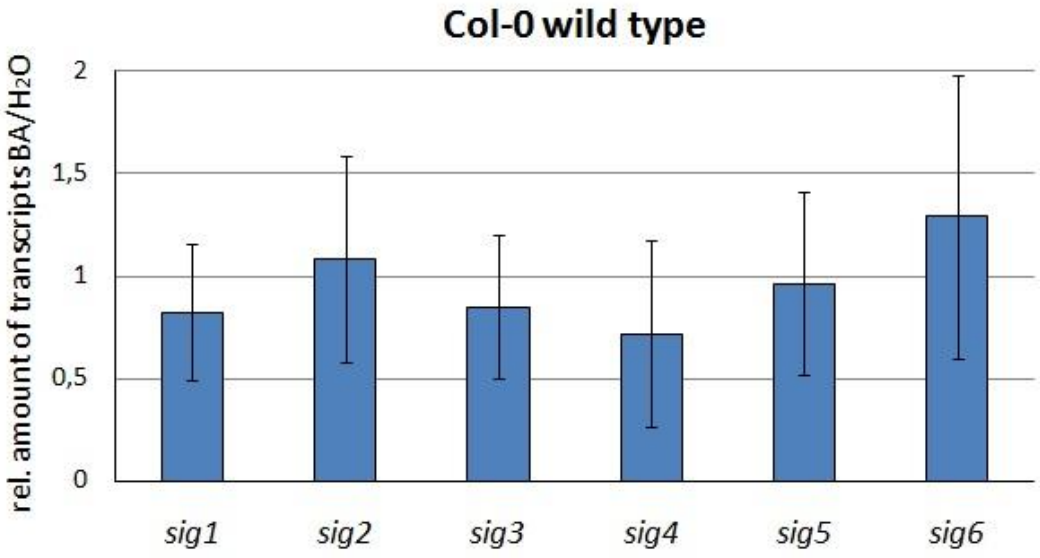

Figure 32: Effect of cytokinin on accumulation of sigma factor gene transcripts in wild type.

Analysis of the accumulation of sigma factor mRNAs (*sig1-sig6*) was done by quantitative real-time PCR. Ratios of the transcript accumulation in cytokinin-treated and non-treated Col-0 wild-type seedlings are presented as means from three independent experiments ± SE. UBQ11 mRNA levels were used as internal standard. Plants were cultivated on top of a net placed on soil. A 24-h pre-incubation of 12-day-old plants on water and subsequent incubation for 6 h on water or BA solution was done.

4 Discussion

The regulation of organellar gene transcription is not only subject to post-transcriptional events (Barkan and Goldschmidt-Clermont, 2000; Monde *et al.*, 2000; Stern *et al.*, 2010), also exogenous and endogenous factors such as light, temperature, hormones, plastid type and chloroplast development possess effects on transcription of distinct organellar genes (Rapp *et al.*, 1992; Mullet, 1993; Mayfield *et al.*, 1995; Link, 1996; Liere and Börner, 2007 a,b). In this work, two of these parameters were at the focus of extensive analyses. Both, the effect of different light wavelengths on the transcript levels of organellar RNA polymerases as well as the effect of the plant hormone cytokinin on plastidial gene transcription, were assessed experimentally.

4.1 Influence of light on *RpoT* transcript levels

Light acts as an environmental signal to adjust plant growth and development (Casal *et al.*, 2004), but also plays an important role in activating plastid transcription in higher plants (Liere *et al.*, 2011). The expression of a number of plastid and nuclear encoded genes involved in photosynthesis is regulated by light and additionally the expression of genes encoding mitochondrial proteins (*e.g.* components of the respiratory chain) is regulated in a light and/or circadian-dependent manner (Yoshida and Noguchi, 2011). As sessile organisms, plants have evolved a number of different photoreceptors for the response to light in their environment (Chory, 2010). Most important photoreceptors include the red light absorbing phytochromes (Quail *et al.*, 1995) and the blue light absorbing cryptochromes (Cashmore *et al.*, 1999; Lin and Shalitin; 2003). A specific photoreceptor for green light has yet to be identified (Folta and Maruhnich, 2007).

Arabidopsis thaliana possesses three different nuclear-encoded phage-type RNA polymerase genes (*RpoT*). The gene products are imported into plastids (RpoTp), mitochondria (RpoTm) or are dual-targeted (RpoTmp). Apart from RpoTp and RpoTmp (NEP), plastids contain an additional plastid-encoded polymerase (PEP). The plastidial gene *rpoB* codes for the beta-subunit of the PEP, but has been reported to be solely transcribed by NEP (Swiatecka-Hagenbruch *et al.*, 2007, 2008; Courtois *et al.*, 2007). Both NEP and PEP contribute to the transcriptional activity in plastids, but NEP activity predominates during early developmental stages (Hajdukiewicz *et al.*, 1997).

Dark-grown etiolated *Arabidopsis* seedlings were used to study the specific transcript accumulation of *RpoT* and *rpoB* genes under different light regimes using quantitative real-time PCR. To test the light system used, responses of known light-regulated transcripts under specific light conditions were analyzed. As expected, light-responsive genes such as *AIP* (red; Tepperman *et al.*; 2004), *cab1* (blue; Hamazato *et al.*, 1997) and *elip1* (green; Dhingra *et al.*, 2006), were strongly light-induced (Figure 8).

4.1.1 White light: differential stimulation of *RpoT* gene expression

Preuten (2010) has shown that steady-state transcript levels of all *RpoT* genes increased when wild-type plants of the Col-0 background were illuminated with white light. The present study was focused on the different light qualities and their receptors involved in the observed activation of *RpoT* transcript accumulation by light. Therefore, one strategy was to use known photoreceptor mutants to reveal their role in the light regulation of *RpoT* expression. However, the photoreceptor mutants used in this study have a Landsberg *erecta* (L*er*) background. It is known from other publications that distinctions between different ecotypes exist (Chevalier *et al.*, 2004; Huang *et al.*, 2010). To exclude ecotype-dependent variations, changes of *RpoTm*, *RpoTmp* and *RpoTp* transcript levels in the L*er* wild-type plants were also analyzed. A light-induced transcript accumulation for all *RpoT* genes within six hours after light exposure was found (Figure 9). The accumulation of *RpoTp* transcripts was affected more than those of *RpoTm* and *RpoTmp*. Compared to data of Preuten (2010), the *RpoT* transcript accumulation in L*er* wild-type seedlings was similar to the one found in Col-0 wild-type seedlings. As a result, ecotype-related variations of the light-inducible *RpoT* gene expression could be excluded.

In addition, the accumulation of *rpoB* gene transcripts was analyzed to determine if the induction of *RpoT* genes by white light may be also associated with an enhanced expression of plastidial genes. It was shown that steady-state transcript levels of the plastidial *rpoB* gene increased when plants were illuminated with white light (Figure 23A). A steady increase in transcripts of *rpoB* was seen up to twelve hours of illumination. The results indicate that enhanced transcript levels of *RpoT* genes translate into higher NEP activity in plastids and might be responsible for the later increase of *rpoB* transcripts. These observations are in agreement with previous experiments in barley that showed the amount of NEP-transcribed plastid genes, including *rpoB*, to follow the pattern of *RpoTp* transcript accumulation (Emanuel *et al.*, 2004).

Interestingly, during the exposure to red, blue and green light no induction of *rpoB* gene expression was detected (Figure 23B-D). As mentioned earlier the *rpoB* operon is known to be transcribed by NEP. In tested monochromatic red, blue and green light conditions the presence of RpoTp alone may not be sufficient to activate the transcription of the *rpoB* operon and may need additional factors. Liere and Börner (2007 a,b; 2011) propose a model where NEP may need additional, yet unknown protein factors for promoter recognition. These unknown factors may need the entire spectrum of light to activate light induction of *rpoB* gene expression, as was seen after twelve hours of white light treatment.

Recent studies in *Arabidopsis* showed that RpoTp plays a major role in transcription and biogenesis of the chloroplast (Hricová *et al.*, 2006), while RpoTmp is supposed to be mainly active in non-green tissue (Emanuel *et al.*, 2006). Recently, Kühn *et al.* (2009) could show that RpoTmp performs gene-specific transcription in mitochondria. Experiments with *rpoTp* mutants showed that RpoTmp is capable to at least partially substitute for RpoTp function (Courtois *et al.*, 2007; Swiatecka-Hagenbruch *et al.*, 2008). Preuten (2010) proposed that RpoTp is the key enzyme during light-induced plastidial transcription in etiolated seedlings, while Baba *et al.* (2004) implicated that RpoTmp plays a major role immediately after the start of chloroplast development during de-etiolation. The results in the present study are in accordance to the data of Preuten (2010), since the *RpoTp* transcript level showed the strongest induction by light in *Arabidopsis* L*er* wild-type seedlings again underlying its important role in plastidial transcription during de-etiolation.

It has been often reported in the literature that the plastid developmental status affects the expression of NEP and PEP as well as the expression of their plastidial target genes in higher plants. For example, experiments in maize (Cahoon *et al.*, 2004) and barley (Emanuel *et al.*, 2004) could show that PEP-derived transcript levels increased as proplastids developed into chloroplasts. While RpoTp becomes less abundant as plastids mature, the mRNA levels of NEP-transcribed genes were found to be stable during plastid development (Cahoon *et al.*, 2004). Emanuel *et al.* (2004) suggested that high RpoTp transcript levels are needed to build up the plastid machinery for transcription (*e.g.* represented by *rpoB*; gene for PEP-subunit) and translation (*e.g.* represented by *rpl2*; gene for ribosomal protein of the large subunit) during the transition from proplastids to chloroplasts. Therefore, the influence of the plastid developmental status on the expression and activity of nuclear genes, including the phage-type RNA polymerases, might involve specific signal transfer between the organelles, *e.g.* plastid-to-nucleus signaling (Emanuel *et al.*, 2004; Woodson and Chory, 2008).

Taken together, all *RpoT* genes are light-inducible by white light, but to a different extent. Light-induced control of plastidial gene transcription mainly *via RpoTp* is important to ensure a rapid build-up of the photosynthesis apparatus in etiolated seedling probably using plastid-to-nucleus signaling. The perception of light signals is mediated by different photoreceptors and involves complex pathways to modulate gene expression in response to light. To gain more information about the role of different photoreceptors in the signaling cascade, L*er* wild-type seedlings and photoreceptor knockout mutants were exposed to monochromatic red, blue and green light.

4.1.2 Red light: two classes of photoreceptors important for *RpoT* genes

In the wild-type plants an increase of all *RpoT* transcripts was found within six hours of illumination with red light (Figure 10). The *Rpo*T transcript accumulation was altered in knockout mutants for the red light absorbing phytochromes. In *phyA* and *phyB* single mutants as well as in *phyA/phyB* double mutants only *RpoTp* transcript accumulation was induced by red light (Figure 11). However, overall effects were less pronounced than in the wild type. Also changes in the *RpoT* transcript amounts were analyzed in knockout mutants for the blue light absorbing cryptochromes. In *cry1* and *cry2* single mutants, and *cry1/cry2* double mutants, the transcript accumulation of *RpoT*s was strongly inhibited (Figure 12). The data support a model in which phytochromes and cryptochromes account for the perception of red light signals, because any missing photoreceptor resulted in a decrease of *RpoT* transcripts (Figure 33).

Cryptochromes may possess additional blue light independent activities in the presence of red light and far-red light. The data suggest that cryptochromes can detect and even mediate red light signals, which led to an activation of *RpoT* transcript accumulation. Several blue light independent cryptochrome actions were reported before (see review by Yu *et al.* 2010). For example, Más *et al.* (2000) reported in a study about functional interaction between phytochrome B and cryptochrome 2 that *cry2* mutant seedlings showed a longer period length of the circadian clock in red light. It is also known that cryptochromes may interact with phytochromes in the absence of blue light (Ahmad *et al.*, 1998b; Más *et al.*, 2000; Yu *et al.*, 2010). A synergistic interaction of red and blue light in the control of *Arabidopsis* gene expression and development was reported by Sellaro *et al.* (2009). More recently, Peschke and Kretsch (2011) showed that PHYA and cryptochromes are concomitantly involved in the regulation of gene expression under red and blue light in *Arabidopsis* seedlings. Experiments

by Strasser *et al.* (2010) with a quintuple phytochrome mutant demonstrated that *Arabidopsis thaliana* mutant seedlings indeed show detectable greening under red light. The red light signal seems to be detected *via* photoreceptors other than phytochromes. Together, the results indicate that under certain conditions cryptochromes are able to replace phytochrome function in red light signaling.

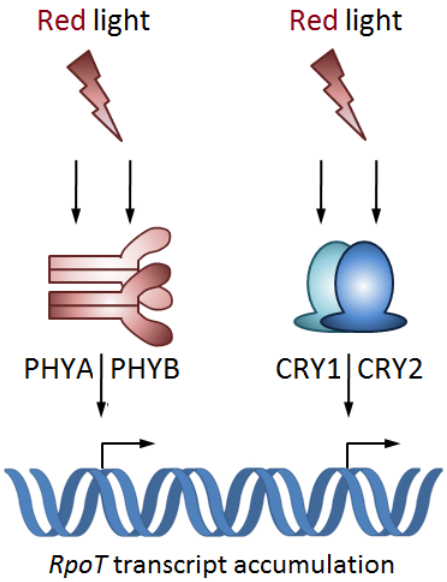

Figure 33: Involvement of photoreceptors in the *RpoT* transcript accumulation (red light).

Based on the data obtained in the different experiments in this study a model was developed that shows the involvement of photoreceptors in red light induced *RpoT* transcript accumulation. PHYA, PHYB, CRY1 and CRY2 account for the perception of red light signals. An arrow indicates a positive effect on transcript accumulation.

4.1.3 Blue light: CRY-mediated down-regulation of *RpoT* gene expression

Functional interaction between phytochromes and cryptochromes has been reported in various light responses such as the control of floral initiation (Guo *et al.*, 1998; Mockler *et al.*, 1999), activation of chloroplast transcription (Chun *et al.*, 2001; Thum *et al.*, 2001) and recently, modulation of hypocotyl phototropism (Tsuchida-Mayama *et al.*, 2010). Phytochromes are known mainly for their function as red light photoreceptors but they are able to absorb a wide spectrum of different wavelengths, including blue light (Schafer and Haupt, 1983; Shinomura *et al.*, 1996; Folta and Spalding, 2001; Spalding and Folta 2005).

Interestingly, blue light was found to have no significant effect on the expression of *RpoT* genes in the wild type (Figure 15). Compared to the wild type, in the *phyA* mutants as well as in the *phyB* mutants, a very weak light induction, mainly for *RpoTp* transcripts, was

detectable (Figure 16). The *phyA/phyB* double knockout mutants showed no significant difference to the wild type. In contrast to the *RpoT* transcript abundance in the wild type, *cry1* and *cry2* single knockout mutants showed an increase for the *RpoTp* transcripts (Figure 17). Furthermore, the strongest increase for *RpoTp* was found for the *cry1/cry2* double mutants.

Despite its role as a potential sensor of red light, the data indicate that indeed cryptochromes mediate blue light signals involved in the *RpoT* gene expression. However, the monochromatic blue light perceived *via* cryptochromes seems to be involved in muting the light activation of *RpoT*s. A disruption of the signal pathway *via* knockout of the cryptochromes apparently cancels the nullifying effect of blue light on the *RpoT* transcript accumulation. Most likely, blue light recognized by a different kind of photoreceptors is, in the absence of cryptochromes, able to induce *RpoT* transcript accumulation. This is in accordance to recent findings by Peschke and Kretsch (2011), who demonstrated cryptochromes to be dispensable during the early-light regulated transcription of marker genes like HY5 by blue light. These data additionally suggest that blue light may be sensed by other photoreceptors, *e.g.*, phytochromes or phototropins.

A role of PHYA in the blue light response has been previously reported (Casal and Mazzella, 1998; Neff and Chory, 1998; Poppe *et al.*, 1998). The data presented here cannot completely exclude an involvement of phytochromes in blue light induced *RpoT* transcript accumulation in *cry* mutants. The knockout of phytochromes in the presence of cryptochromes would not lead to a decrease of *RpoT* transcripts, since the transcript accumulation is already repressed. In the proposed model, blue light recognized by phytochromes would be able to induce *RpoT* transcript accumulation only in the absence of cryptochromes (Figure 34). To test this hypothesis and to clarify the precise function of each of the (possible) blue light photoreceptors, analysis of combinatorial multiple mutants might be necessary. The next step would be to analyze the effect of blue light on the *RpoT* transcript accumulation in a quadruple mutant (c*ry1/cry2/phyA/phyB*) and corresponding triple mutants, by crossing the *cry1/cry2* mutant with the *phyA/phyB* mutant. Similar experiments with combinatorial multiple mutants in *Arabidopsis* revealed for example that phototropins only play a minor role in blue light induced gene expression and are more likely involved in mediating photomovement responses (Ohgishi *et al.*, 2004). However, it cannot be ruled out that in *cry* mutants phototropins or other yet to be identified photoreceptors are responsible for the observed activation of *RpoT* transcript accumulation.

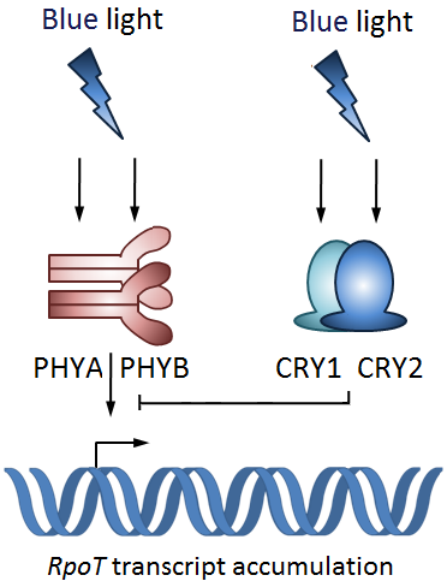

Figure 34: Involvement of photoreceptors in the *RpoT* transcript accumulation (blue light).

Based on the data obtained in the different experiments in this study a model was developed that shows the involvement of photoreceptors in blue light repressed *RpoT* transcript accumulation. CRY1 and CRY2 account for the perception of blue light signals. In addition, blue light may be recognized by other photoreceptors like phytochromes. The blue light signals perceived by these receptors are somehow repressed by cryptochromes and induce *RpoT* transcript accumulation only in the absence of cryptochromes. An arrow indicates a positive effect on transcript accumulation, while a line with a blunt end indicates an inhibitory effect.

Several microarray studies have been performed to investigate the influence of blue light on the organellar gene expression. Ma *et al.* (2001) used expressed sequence tag-based (EST) microarrays to study light control on the genome expression in 6-day-old *Arabidopsis* wild-type seedlings. 1096 ESTs were induced at least twofold by blue light, but more interestingly, 616 ESTs were repressed under the same light quality. Their results correspond to recent microarray experiments by Zhang *et al.* (2008) that showed 123 genes to be induced and 97 genes to be repressed by blue light in a CRY1-dependent manner. For example, after one hour of blue light exposure *RpoTp* gene expression was slightly enhanced in *cry1* mutants compared to wild-type seedlings. Together, these data suggest that down-regulation of transcript levels by blue light seems to be a more common aspect. A potential role of the specific blue light effect on *RpoT* transcript accumulation under natural conditions remains to be elucidated.

4.1.4 Green light: *RpoT* transcripts regulated *via* phytochromes and CRY2

The exposition to green light showed a very interesting accumulation pattern for the wild type (Figure 19). A strong increase of all three *RpoT* transcript amounts after four hours of illumination was detectable, while further light exposure led to a decrease of transcripts back to levels found in darkness. The knockout of one or two phytochromes led to reduction of the transcript amounts compared to the wild type (Figure 20). In the *cry1* single knockout mutants the peak for the *RpoT* genes was just shifted compared to the wild type (Figure 21). In both *cry2* and *cry1/cry2* mutants no significant increase of *RpoTm* and *RpoTmp* transcript levels was detectable. In contrast, *RpoTp* transcript amounts were increased. According to the data, a model in which PHYA, PHYB and CRY2 contribute in the regulation of the *RpoT* transcript accumulation under green light is proposed (Figure 35).

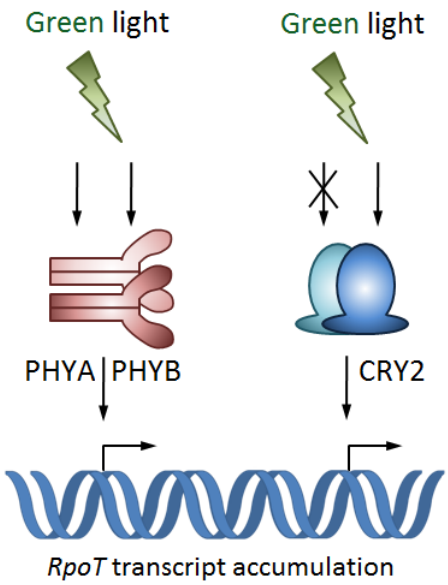

Figure 35: Involvement of photoreceptors in the *RpoT* transcript accumulation (green light).

Based on the data obtained in the different experiments in this study a model was developed that shows the involvement of photoreceptors in green light induced *RpoT* transcript accumulation. PHYA, PHYB and CRY2 account for the perception of green light signals. An arrow indicates a positive effect on transcript accumulation, while a crossed out arrow indicates no light perception *via* a certain photoreceptor.

The detection of green light signals *via* cryptochromes and phytochromes may be of advantage for the optimization of light capture under unfavorable growth conditions (Folta, 2004; Folta and Maruhnich, 2007). Furthermore, Dhingra *et al.* (2006) proposed the existence of a hypermorphogenetic state in green light, which allows the plant to reach better light conditions through preservation of needed resources by the plastids. The biological role of

enhanced *RpoT* transcript levels after four hours of illumination with green light has to be further investigated. Despite this significant peak, the *RpoT* transcript accumulation in wild type was in the range of dark control levels, which might lead to reduction of overall plastid transcription. The proper development of chloroplasts might be affected and energy could be saved for other processes.

4.1.5 HY5: central signal integrator in all tested light conditions

The transcription factor HY5 acts downstream of photoreceptors like phytochromes and cryptochromes (Koornneef *et al.*, 1980; Oyama *et al.*, 1997; Ang *et al.*, 1998; Ulm *et al.*, 2004). Absent in darkness, it accumulates rapidly upon exposure to light and regulates the transcription of light-responsive genes (Ang *et al.*, 1998; Chattopadhyay *et al.*, 1998; Osterlund *et al.*, 2000). Furthermore, HY5 promotes photomorphogenesis in red, far-red and blue light conditions (Lau and Deng, 2010). Therefore, the *RpoT* transcript accumulation in *hy5* mutants was additionally analyzed under red, blue and green light conditions.

The present study confirmed the role of HY5 as a central signal integrator under all different light conditions for nuclear-encoded RNA polymerases. In HY5 deficient plants the expression of the *RpoT* genes was strongly affected compared to the wild-type results, suggesting that HY5 is important for the transfer of light signals to the *RpoT*s. As mentioned before, in red and green light a drastic decrease of *RpoT* transcripts in *hy5* mutants was detectable (Figure 14+Figure 22), while blue light mediated inhibition was interrupted in *hy5* mutants (Figure 18). For example, in *hy5* mutants an increase of transcripts after illumination with blue light was found, similar to what had been observed in cryptochrome knockout mutants. This suggests HY5 plays a major role downstream of cryptochromes in mediating blue light signals in repressing *RpoT* gene expression. In addition, HY5 may have a large influence on photoreceptor function itself. For instance, it was recently shown to be involved in the negative feedback regulation of PHYA signaling (Li *et al.*, 2010). The important role of HY5 as a central integrator of light and hormones as well as in light-regulated plant development is known from the literature (Lee *et al.*, 2007; Lau and Deng, 2010). For example, more than 60% of early PHYA- or PHYB-induced genes are targets of HY5 (Lee *et al.*, 2007) To summarize, the data presented indicate that HY5 seems to be involved in both cryptochrome- and phytochrome-mediated effects on light-induced *RpoT* gene transcript accumulation under different light conditions.

4.1.6 Summary: light effects on organellar RNA polymerases

Many light-induced processes require a complex regulation on multiple levels and through several interconnected pathways (Casal, 2000; Eisinger *et al.*, 2003; Casal and Yanovsky, 2005; Jiao *et al.*, 2007; Peschke and Kretsch, 2011). In the present study, the focus lay on processes which occurred after illumination of etiolated 7-day-old *Arabidopsis thaliana* seedlings with different light qualities. Light-regulated processes during this developmental stage include seed germination and seedling photomorphogenesis. In *Arabidopsis* seedlings, photomorphogenesis is characterized by inhibition of hypocotyl growth, cotyledon opening and expansion, and the synthesis of the photosynthetic apparatus (Casal *et al.*, 2003; Yu *et al.*, 2010). Under natural conditions (sunlight) the light spectrum ranges from UV-B to the far-red, but different wavelengths can have specific effects during plant growth and development. For example, red light promotes seed germination (Casal *et al.*, 2003), whereas blue light inhibits hypocotyl elongation (Yu *et al.*, 2010). For the effective initiation of de-etiolation in higher plants the exposure to both red and blue light signals is essential. Light-induced changes of the organellar gene transcription which involves interactions of several photoreceptors might support these morphological and physiological changes.

Several light-induced effects on the transcript levels of organellar RNA polymerases were shown in the present study. Rapid induction of *RpoT* transcript accumulation by red and blue light might be important for the fast adaptation of seedlings to light after growth in the darkness. Light-induced control of organellar gene expression and a fast build-up of the photosynthesis apparatus in etiolated seedling are important during light adaptation. It is often mentioned in the literature that the expression of many rapidly responding genes is regulated by red and blue light (Tepperman *et al.*, 2006; Peschke and Kretsch, 2011). Green light signals are often discussed as acting antagonistic to red and blue light signals (Folta and Maruhnich, 2007). It might be possible that green light is sensed as an environmental stress signal. As sessile organisms plants need to optimize light capture under unfavorable growth condition. Despite a distinct peak, the *RpoT* transcript accumulation was not induced by green light. Small amounts of NEP might lead to a reduction of overall plastid transcription, which saves energy needed to reach better light conditions.

The entire spectrum of light is required for a correct light regulation of organellar genes. Under natural light conditions the contribution of different monochromatic light qualities may vary. This would explain the strong increase of *RpoT*s in white light, which is not seen under monochromatic blue or green light. The cross-talk between different photoreceptors is important to adjust the organellar transcription to natural light conditions. It is known that several synergistic and antagonistic interactions between phytochromes and cryptochromes exist in the control of plant growth and development (Casal and Mazzella, 1998; Casal, 2000). Therefore it would not be surprising if these interactions also influence the *RpoT* transcript accumulation.

On one hand, an explanation for the observed results could be, that the same interactions of blue and red light, which are essential for seedling germination and development, are responsible for enhancing the expression of numerous genes including *RpoT*s in white light. On the other hand it was found that monochromatic red light induced *RpoT* transcript accumulation, while in experiments with monochromatic blue light no induction of *RpoT* transcripts occurred. As mentioned before phytochromes are able to detect red and blue light signals (see 4.1.3). Thus, another hypothesis would be that the blue light sensing activity of phytochromes was somewhat impaired by antagonistic cryptochrome activity to ensure that phytochromes perceive under natural light conditions primarily red light signals to promote *RpoT* transcript accumulation during early seedling development (Figure 36). Higher level of *RpoT* transcripts might translate into higher level of needed NEP-transcribed genes.

The present study showed that light-induced regulation of *RpoT* gene expression is not only dependent on one class of photoreceptors, but utilizes the whole spectrum of phytochromes and cryptochromes to adjust gene expression in the natural environment. Additionally, analyzing effects of different light qualities on transcript levels of organellar genes transcribed by nuclear-encoded RNA polymerases *via* microarray analysis will be very interesting. Run-on analyses could provide more information about transcript stability and *de novo* synthesis.

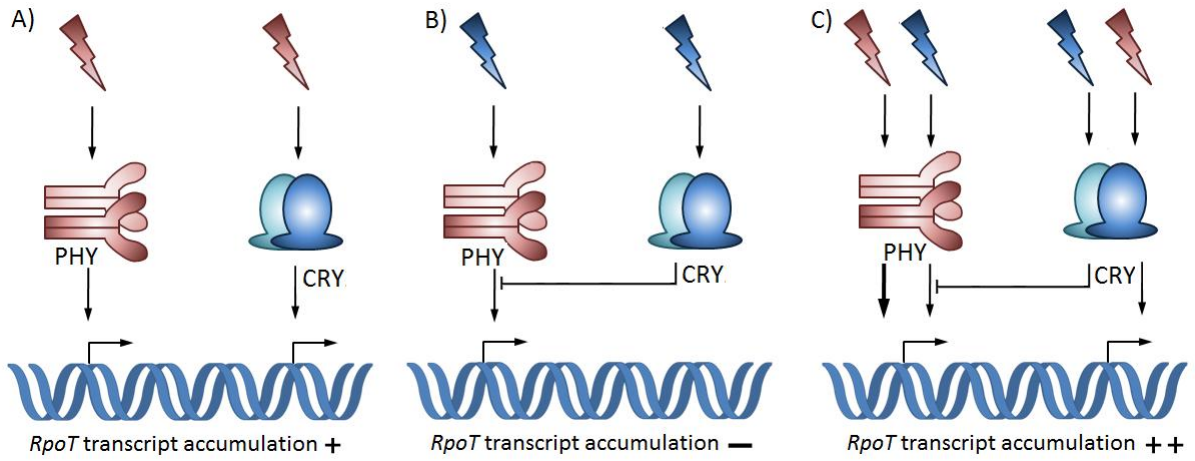

Figure 36: Model for the regulation of *RpoT* gene transcript accumulation by red and blue light.

Based on the data obtained in the different experiments in this study a model was developed that shows the involvement of phytochromes (PHY) and cryptochromes (CRY) in red and blue light regulated *RpoT* transcript accumulation. Monochromatic red light induced *RpoT* transcript accumulation (A), while in monochromatic blue light *RpoT* transcripts were repressed (B). It was hypothesized that the blue light sensing activity of phytochromes was somewhat impaired by antagonistic cryptochrome activity to ensure that phytochromes perceive under natural light conditions primarily red light signals to promote *RpoT* transcript accumulation (C). An arrow indicates a positive effect (+) on transcript accumulation, while a line with a blunt end indicates an inhibitory effect (-). Thickness of the lines corresponds to the importance of the respective receptor.

4.2 Influence of cytokinin on the organellar gene transcription

The plant hormone cytokinin regulates many aspects of plant growth and development, including de-etiolation, circadian clock oscillation, chloroplast differentiation, root and shoot related processes, and responses to environmental stimuli (reviewed by Mok and Mok, 2001; Zheng *et al.*, 2006; Kurakawa *et al.*, 2007; Argueso *et al.*, 2009; Werner and Schmülling, 2009). Recent data showed that the application of cytokinin increases the transcription of some plastidial genes in barley, tobacco and *Arabidopsis thaliana* (Brenner *et al.*, 2005; Zubo *et al.*, 2008; Hertel, 2009). The responses of plastids could result indirectly from inducing the expression of some nuclear-encoded components of the plastid transcription machinery such as RNA polymerases or sigma factors. Another option could be that cytokinin may also directly affect the transcription apparatus of plastid genes *via* special signal transduction pathways. To gain additional information about the role of different hormone receptors and response regulators in plastid gene expression, the response to cytokinin in Col-0 wild-type seedlings and cytokinin-pathway-related knockout mutants was analyzed on several levels. In addition, cytokinin-deficient *CKX1* mutants were used to study the influence of the endogenous cytokinin pool on the responsiveness to exogenous cytokinin.

4.2.1 Cytokinin application led to more, but smaller chloroplasts

To study which effects the addition of exogenous cytokinin has on the plastid division, microscopic analysis of chloroplast parameters like size and number were performed in Col-0 wild-type plants and cytokinin-related mutants. In the present data, wild-type plants and most analyzed mutants displayed a larger number of smaller chloroplasts after exogenous application of cytokinin (Figure 25, Figure 26). Exceptions were found for the cytokinin-deficient *CKX1* mutants (no change in chloroplast number, but decreased chloroplast size) and the *ahk2/ahk3* mutants (no change in chloroplast size, but increased chloroplast number).

Mutations in single receptors did not cause strong changes in the cytokinin response, which indicates a high degree of redundancy in the function of these receptors. However, it seems that the CRE1/AHK4 receptor alone was not sufficient to mediate a full response to the cytokinin signal, since the combined loss of AHK2 and AHK3 resulted in no change in the chloroplast size after cytokinin application. The observed results indicate that synergistic effects of the cytokinin receptors AHK2 and AHK3 may be to some extent responsible for the cytokinin-regulated chloroplast division, since only the knockout of both receptors inhibits the effect of cytokinin on the chloroplast size. Cytokinin-deficiency caused a retardation of shoot

development in *35S:AtCKX* transgenic plants (Werner *et al.*, 2003). The lower endogenous cytokinin level in *CKX1* mutants may at least in part be responsible that the effects of exogenously applied cytokinin on the chloroplast number were less pronounced compared to the wild type.

Cytokinin may also influence chloroplast size and numbers *via* CRF transcription factors (CRF: Cytokinin Response Factor) that regulate the gene expression of plastid division components. For example, data by Okazaki *et al.* (2009) showed that exogenously applied cytokinin led, at least in part *via* CRF2, to elevated PLASTID DIVISION (PDV2) protein levels. This resulted in an increase of the number, but a decrease of size of chloroplasts in *Arabidopsis thaliana* (Okazaki *et al.*, 2009). Higher plants can adapt to fluctuating environmental light situations through light-dependent chloroplast movement (see review by Takagi, 2009). The presence of a larger number of smaller chloroplast would be an asset when plants have to grow under varying light conditions. A recent study in tobacco showed that a large population of small chloroplast allows more effective chloroplast phototaxis, which allows efficient utilization of energy under dim light and minimization of photodamage caused by excess light (Jeong *et al.*, 2002).

4.2.2 Cytokinin application increases plastome copy numbers

The plastid genome (plastome) of a typical higher plant is 120-160 kb in size and contains about 120 genes (Sugiura, 1992; Wakasugi *et al.*, 2001). In a diploid plant cell only two copies of the nuclear genome, but up to thousands of copies of the plastome are present (Zoschke *et al.*, 2007). Since most plastome-encoded gene products are involved in photosynthesis, adjusting copy numbers to changing demands might offer a potential for regulating chloroplast gene expression (Li *et al.*, 2006). However, Zoschke *et al.* (2007) showed that plastidial gene expression is not regulated by plastome copy number, but rather at the transcriptional and post-transcriptional levels. Another theory for high plastome copy numbers is that they are needed to provide sufficient ribosomes for the developing plastid *via* genome amplification (Bendich, 1987; Rogers *et al.*, 1993). Using quantitative real-time PCR, plastome copy numbers in wild type and cytokinin-related mutants were calculated and adjusted to the nuclear *RpoTm* gene copy numbers and the degree of nuclear genome polyploidization. This was done, because nucleoids can have different quantities of DNA and the degree of their ploidy can vary. The development of the photosynthetic apparatus in plants is associated with an increase of chloroplast numbers. Therefore, endopolyploidization has

been discussed as an option to adapt the number and expression of nuclear-encoded plastid proteins to these changing conditions (Galbraith *et al.*, 1991).

Average nuclear ploidy numbers (Figure 27) and plastome copy numbers (Figure 28) increased slightly in Col-0 wild type and nearly all cytokinin-related mutants after application of cytokinin. Furthermore, the cytokinin receptor single (*cre1*) and double mutants (*ahk2/ahk3*) showed no significant change in their nuclear ploidy levels and in their plastome copy numbers after BA treatment suggesting that cytokinin reception is indeed the reason for increasing values. The simple combination of AHK2 and AHK3 (*cre1* mutants) as well as CRE1 alone (*ahk2/ahk3* mutants) was not sufficient to transmit the cytokinin signal. However, the combination of AHK2 and CRE1 as well as of AHK2 and CRE1 together provided full cytokinin responsiveness. The presence of an increased number of smaller chloroplast in response to cytokinin treatment might influence the plastome copy numbers per cell. Previous findings in *Zea* species (maize; Oldenburg and Bendich, 2004; Zheng *et al.*, 2011) and *Beta vulgaris* (sugar beet; Rauwolf *et al.*, 2010) suggest that the plastome copy number is at least in part dependent on plastid size and number. On the other hand, a direct influence of cytokinin on the plastome copy numbers cannot be excluded and should be further investigated.

4.2.3 All three receptors participate in regulating the plastid gene transcription

The regulation of the plastidial gene transcription by cytokinin in Col-0 wild-type plants and cytokinin-related mutants was studied using chloroplast isolation and run-on transcription assays. In *Arabidopsis* wild-type plants, which were grown on medium with cytokinin, the transcription in chloroplasts most likely adapted to the permanent high cytokinin levels (Figure 29). No significant hormone response was found compared to seedlings grown without cytokinin. In contrast, chloroplast gene transcription in wild-type plants grown on soil without cytokinin showed a significant light-dependent response to cytokinin after incubation for six hours (Figure 30). Housekeeping genes are required for the maintenance of basic cellular function, while photosynthesis-related genes encode proteins which are needed in photosynthetically active chloroplasts. Therefore, it was not surprising that the activation of transcription of housekeeping genes by cytokinin was slightly stronger than those of photosynthesis-related genes.

The effects of cytokinin on chloroplast transcription were reduced in all three kinase single mutants, although more prominent in *ahk3* and *cre1* than in *ahk2*. In double mutants the cytokinin effects were even more reduced than in the single mutants. The results indicated that the three histidine kinase receptors play essential, but functionally redundant roles in the cytokinin signaling pathway of *Arabidopsis thaliana*. Mutations in the *cre1* and the *ahk3* genes stronger abolished cytokinin action on chloroplast gene transcription then a mutation in the *ahk2* gene. This is in accordance to several reports that showed AHKs to be functionally overlapping regulators in cytokinin signaling (Higuchi *et al.*, 2004; Nishimura *et al.*, 2004; Riefler *et al.*, 2006). It was suggested by Ishida *et al.* (2009) that ARR1, ARR10, and ARR12, together play essential but redundant roles in cytokinin signal transduction in *Arabidopsis thaliana.* In contrast, the results presented indicate a more prominent role of ARR1 in the cytokinin signal transduction pathway, since a knockout of *ARR1* resulted in similar transcript accumulation pattern as observed in single receptor mutants.

A decrease of chloroplast gene transcripts was also found in *CKX1* mutants, which overexpress cytokinin-degrading CKX1 enzymes. This indicates that the internal cytokinin content might be an important signal for regulating the plastidial transcription maybe *via* restriction of the signal transduction pathway. Interestingly, microarray analysis showed that the cytokinin receptor gene *CRE1* was down-regulated in *35S:AtCKX1* transgenic seedlings (Brenner, 2005). Cytokinin-deficient plants might use a sensitized signaling system to compensate for the lower internal cytokinin content (Brenner, 2005), but the overall response of the transgenic *CKX1* mutants was reduced compared to the *Arabidopsis* wild type. In contrast, experiments with transgenic *Nta:AtCKX2* tobacco showed an enhanced sensitivity for cytokinin (Hertel, 2009). These findings indicate that the cytokinin effects on plastid transcription depending on endogenous cytokinin can vary in different plant species.

4.2.4 Sigma factors are involved in the cytokinin-regulated gene transcription

Bacteria use a set of sigma factors to regulate their gene transcription. Sigma factors are cofactors that enable specific binding of RNA polymerase to gene promoters (Wösten, 1998; Schweer, 2010). They have also been found in several plant species, including *Arabidopsis*, maize, rice, wheat, and tobacco (Lysenko, 2007; Schweer, 2010). Six different sigma factors exist in *Arabidopsis*, which are activated in response to several environmental conditions. Although thought to have mostly overlapping roles in regulating plastid gene expression by PEP, some sigma factors were shown to exhibit developmentally timed gene specific

function. For instance, the promoters of *rbcL* and *psbA* are recognized by SIG2 early in seedling development, whereas stress-induced SIG5 is essential for the blue light induced transcription of the *psbD* operon (Lerbs-Mache, 2011). Some cytokinin-inducible genes in this study were transcribed *via* the plastid encoded RNA polymerase (PEP), which needs sigma factors for the correct promoter recognition. To investigate the role of sigma factors in the cytokinin signaling pathway, the influence of cytokinin on chloroplast transcription was analyzed in *Arabidopsis* sigma factor mutants by run-on assays (Figure 31) and quantitative real-time PCR (Figure 32).

In wild-type plants an induction of chloroplast gene transcription was found, while the knock-out of distinct sigma factors led to reductive effects. Cytokinin effects on total chloroplast transcription were slightly reduced in *sig3*, *sig4*, and *sig5* mutants and significantly reduced in *sig1*, *sig2*, and *sig6* mutants. Together, the data indicated that some sigma factors like SIG2 and SIG6 might be more involved in cytokinin-induced transcriptional changes than others. According to recent reviews, SIG2 and SIG6 are major factor in early development and may be considered as ‗‗essential for vital plastid functions'' in *Arabidopsis thaliana* (Schweer, 2010; Lerbs-Mache, 2011). Not surprising, SIG2 and SIG6 knock-out plants display a chlorophyll-deficient phenotype (Shirano *et al.*, 2000; Ishizaki *et al.*, 2005; Loschelder *et al.*, 2006).

Regulation of plastid genome transcription *via* sigma factors potentially occurs at several levels (Lerbs-Mache, 2011). Differential expression of the sigma factors themselves provides one means in regulating plastidial gene expression. However, the modulation of sigma factor activity and/or specificity by post-transcriptional modifications such as phosphorylation and redox state is more common (Pfannschmidt und Liere, 2005; Shimizu, 2010). These mechanisms have probably evolved to shorten the signal transduction pathway to respond rapidly to changing environmental conditions (Lerbs-Mache, 2011). The present study showed no effect of cytokinin on the steady-state levels of transcripts of the six sigma factor genes in wild-type seedlings suggesting activation of PEP transcription in response to cytokinin treatment rather by post-translational modifications than activation of sigma-factor expression.

The data suggest an important role of sigma factors in cytokinin-induced chloroplast gene transcription. To further investigate involved pathways microarray analysis and quantitative real-time PCR could be used to analyze target genes of sigma factors and their response to cytokinin. In addition, the detection of SIG2 and SIG6 proteins by Western Blot analysis in

cytokinin-treated seedlings would provide some interesting information about the amount of sigma factors actual present. Post-translational modification of sigma factors could be analyzed by mass spectrometry.

4.2.5 Summary: regulation of plastidial gene transcription by cytokinin

The role of cytokinin receptors and response regulators in the plastid gene expression of Col-0 wild-type seedlings and cytokinin-related mutants was analyzed on several levels. Taken together, the data show certain redundancies within the cytokinin signal perception system. However, the three receptors and their combinations contribute to a different extent to various processes. In accordance to data of Riefler *et al.* (2006), mutation of AHK2 alone did not or only slightly causes a change of cytokinin sensitivity. However, in several experiments, the *ahk2* mutation enhanced the cytokinin resistance of *ahk3* (chloroplast size, ploidy level, plastid gene transcription) or *cre1* (plastid gene transcription) mutants. This indicates that AHK2 may function primarily in combination with AHK3 or CRE1.

The response regulator ARR1 is involved in the cytokinin signal transduction, since in the *ARR1* mutants the plastid gene transcript accumulation was strongly reduced compared to the wild type. The endogenous cytokinin content also influences the plastid gene transcription in response to exogenously applied cytokinin. In cytokinin-deficient *CKX1* mutants, no activation of chloroplast gene transcription by cytokinin was found. The reduction of the cytokinin status, which has been achieved either by lowering the cytokinin content (*CKX1* mutants) or by reducing cytokinin signaling (*ahk* and *ARR1* mutants), led to a decline of specific hormone responses, demonstrating *e.g.* that cytokinin is a positive regulator of plastid gene transcription. In addition, the presented data suggest a regulation of the plastid genome transcription *via* modulation of sigma factor activity and/or specificity by post-translational processes like *e.g.* phosphorylation. SIG2 and SIG6 seem to play an essential role in the cytokinin-response of young seedlings. A possible model for the regulation of chloroplast gene transcription by cytokinin in *Arabidopsis thaliana* is presented in Figure 37.

Cytokinins promote crop productivity for instance by the activation of meristems in rice (Kurakawa *et al.*, 2007) or increasing drought tolerance through suppression of drought induced leaf senescence in tobacco (Rivero *et al.*, 2007). The rate of photosynthesis can be measured indirectly through the rate increase in biomass. An induction of photosynthesis-related genes by cytokinin could lead to an improvement of photosynthesis and an increasing biomass production. For this reason, it is important to gain as much information as possible

about cytokinin signaling cascades and their organellar gene targets for generating stress-resistant crop plants or achieve higher plant biomass for biofuel production. To investigate the influence of cytokinin on photosynthesis and/or light acclimation, analysis of photosystem I and II components like *psaA* and *psbA* on protein and RNA level would be interesting.

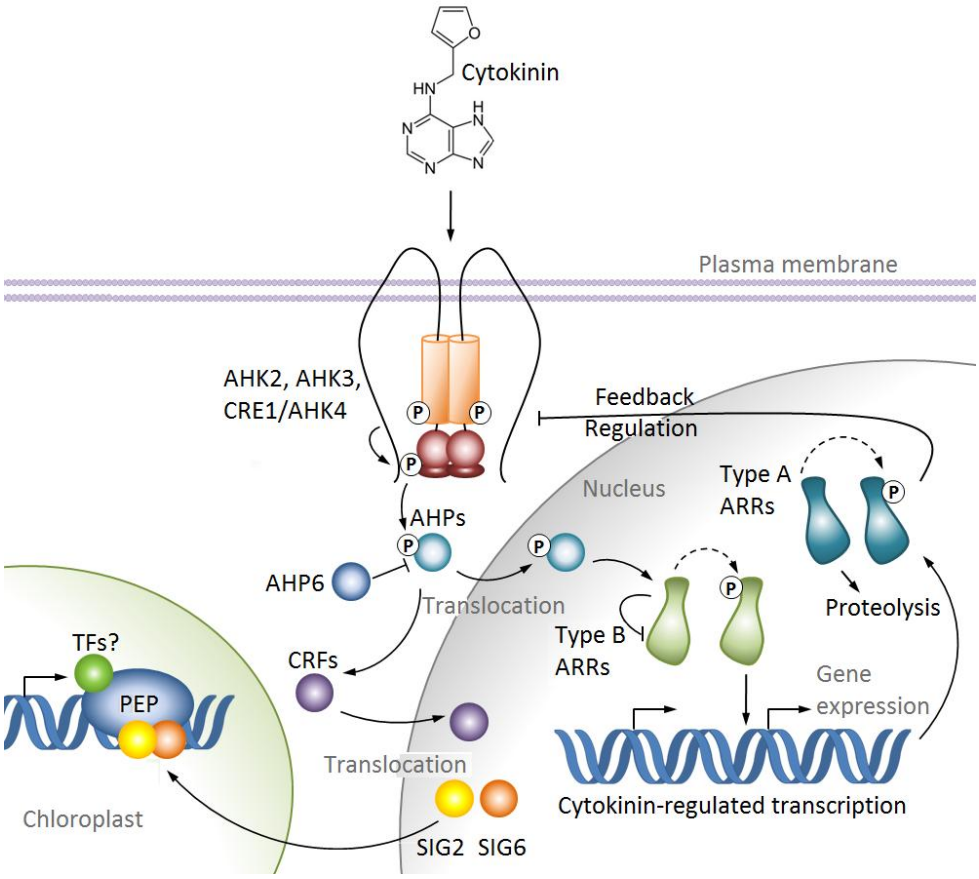

Figure 37: Model for the regulation of chloroplast gene transcription by cytokinin.

Cytokinin treatment leads to a significant induction of chloroplast gene transcription in 12-day-old Col-0 wild type soil-grown on top of a net. Each signaling step in the cytokinin signal transduction pathway is executed by a family of genes that largely act redundantly. Phosphorelay events mediate the hormone signaling from functionally redundant cytokinin receptors (AHK2, AHK3 and CRE1/AHK4) *via* AHP proteins to type-B response regulators (ARRs), which co-activate cytokinin-regulated gene transcription in the nucleus. The CRF proteins are also activated by cytokinin *via* the AHPs to accumulate in the nucleus and activate transcription. The data presented suggests a major role of ARR1 in the cytokinin signaling pathway. How exactly the cytokinin signal is mediated to the chloroplast remains to be further investigated. One possibility would be *via* the transcription machinery of the plastids. The plastid-encoded plastid RNA polymerase (PEP) requires nuclear-encoded sigma factors (SIG) for promoter recognition and additional, yet unknown transcriptional factors (TF) for their correct function. The post-translational modulation of sigma factors, *e.g.* SIG2 and SIG6, may provide one way of cytokinin action on chloroplast transcription. AHK: Arabidopsis Histidine Kinase, AHP: Arabidopsis Histidine Phosphotransfer protein, ARR: Arabidopsis Response Regulator, CRF: Cytokinin Response Factor. Based on Santner *et al.*, 2009.

Bibliography

Ahmad, M. and Cashmore, A.R. (1993): HY4 gene of A. thaliana encodes a protein with characteristics of a blue-light photoreceptor. Nature 366 [*6451*], 162-166.

Ahmad, M., Jarillo, J.A., Smirnova, O. and Cashmore A.R. (1998a): Cryptochrome blue-light photoreceptors of Arabidopsis implicated in phototropism. Nature 392 [*6677*], 720-723.

Ahmad, M., Jarillo, J.A., Smirnova, O. and Cashmore A.R. (1998b): The CRY1 Blue Light Photoreceptor of Arabidopsis Interacts with Phytochrome A In Vitro. Mol Cell 1 [*7*], 939-948.

Allison, L.A. (2000): The role of sigma factors in plastid transcription. Biochimie 82 [*6-7*], 537-548.

Allison, L.A. and Maliga, P. (1995): Light-responsive and transcription-enhancing elements regulate the plastid *psbD* core promoter. EMBO J 14 [*15*], 3721–3730.

Ang, L-H., Chattopadhyay, S., Wei, N., Oyama, T., Okada, K., Batschauer, A. and Deng, X-W. (1998): Molecular Interaction between COP1 and HY5 Defines a Regulatory Switch for Light Control of Arabidopsis Development. Mol Cell 1, 213-222.

Argueso, C.T., Ferreira, F.J. and Kieber, J.J. (2009): Environmental perception avenues: the interaction of cytokinin and environmental response pathways. Plant Cell Environ 32 [*9*], 1147-1160.

Asami, T., Nakano, T. and Fujioka, S. (2005): Plant brassinosteroid hormones. Vitam Horm 72, 479-504.

Azevedo, J., Courtois, F., Hakimi, M-A., Demarsy, E., Lagrange, T., Alcaraz, J-P., Jaiswal, P., Maréchal-Drouard, L. and Lerbs-Mache, L. (2008): Intraplastidial trafficking of a phage-type RNA polymerase is mediated by a thylakoid RING-H2 protein. Proc Natl Acad Sci USA 105 [*26*], 9123–9128.

Baba, K., Schmidt, J., Espinosa-Ruiz, A., Villarejo, A., Shiina, T., Gardeström, P., Sane, A.P. and Bhalerao, R.P. (2004): Organellar gene transcription and early seedling development are affected in the *rpoT;2* mutant of *Arabidopsis*. Plant J 38 [*1*], 38-48.

Baena-Gonzalez, E., Baginsky, S., Mulo, P., Summer, H., Aro, E-M. and Link, G. (2001): Chloroplast Transcription at Different Light Intensities. Glutathione-Mediated Phosphorylation of the Major RNA Polymerase Involved in Redox-Regulated Organellar Gene Expression. Plant Physiol 127 [*3*], 1044-1052.

Banerjee, R. and Batschauer, A. (2005): Plant blue-light receptors. Planta 220 [*3*], 498-502.

Barkan, A. (2011): Expression of plastid genes: organelle-specific elaborations on a prokaryotic scaffold. Plant Physiol 155 [*4*], 1520-1532.

Barkan, A. and Goldschmidt-Clermont, M. (2000): Participation of nuclear genes in chloroplast gene expression. Biochimie 82 [*6-7*], 559-572.

Barow, M. and Meister, A. (2003): Endopolyploidy in seed plants is differentially correlated to systematic, organ, life strategy and genome size. Plant, Cell and Environment 26, 571-584.

Batschauer, A., Banerjee, R. and Pokorny, R. (2007): Cryptochromes. In: Light and Plant Development; Whitelam; G.C, Halliday K.J. (eds.). Blackwell Publishing, Oxford, UK, 17-48.

Baumgartner, B.J., Rapp, J.C. and Mullet, J.E. (1993): Plastid Genes Encoding the Transcription/Translation Apparatus Are Differentially Transcribed Early in Barley (*Hordeum vulgare*) Chloroplast Development. Plant Physiol 101 [*3*], 781-791.

Bendich, A.J. (1987): Why do chloroplasts and mitochondria contain so many copies of their genome? Bioessays 6 [*6*], 279-282.

Benková, E., Witters, E., Van Dongen, W., Kolár, J., Motyka, V., Brzobohaty, B., Van Onckelen, H. A. and Machácková, I. (1999): Cytokinins in tobacco and wheat chloroplasts. Occurrence and changes due to light/dark treatment. Plant Physiol 121 [*1*], 245-252.

Brandstatter, I. and Kieber, J. J. (1998): Two genes with similarity to bacterial response regulators are rapidly and specifically induced by cytokinin in *Arabidopsis*. Plant Cell 10, 1009-1019.

Brenner, W.G., Romanov, G.A., Köllmer, I., Bürkle, L. and Schmülling, T. (2005): Immediate-early and delayed cytokinin response genes of *Arabidopsis thaliana* identified by genome-wide expression profiling reveal novel cytokinin-sensitive processes and suggest cytokinin action through transcriptional cascades. Plant J 44 [*2*], 314-333.

Briggs, W.R. (2007): The LOV domain: a chromophore module servicing multiple photoreceptors. J Biomed Sci 14 [*4*], 499-504.

Briggs, W.R. and Christie, J.M. (2002): Phototropins 1 and 2: versatile plant blue-light receptors. Trends Plant Sci 7 [*5*], 204-210.

Brown, R. and Rickless, P. (1949): A new method for the study of cell division and cell extension with some preliminary observation on the effect of temperature and of nutrients. Proc R Soc Lond B Biol Sci 136 [*882*], 110-125

Cahoon, A.B. and Stern, D.B. (2001): Plastid transcription: a ménage á trois? Trends Plant Sci. 6 [*2*], 45–46.

Cahoon, A.B., Harris, F.M. and Stern, D.B. (2004): Analysis of developing maize plastids reveals two mRNA stability classes correlating with RNA polymerase type. EMBO Rep 5 [*8*], 801-806.

Casal, J.J. (2000): Phytochromes, cryptochromes, phototropin: photoreceptor interactions in plants. Photochem Photobiol 71 [*1*], 1-11.

Casal, J.J: and Mazzella, M.A. (1998): Conditional synergism between cryptochrome 1 and phytochrome B is shown by the analysis of *phyA*, *phyB*, and *hy4* simple, double, and triple mutants in Arabidopsis. Plant Physiol 118 [*1*], 19-25.

Casal, J.J. and Yanovsky, M.J. (2005): Regulation of gene expression by light. Int J Dev Biol 49 [*5-6*], 501-511.

Casal, J.J., Luccioni, L.G., Oliverio, K.A. and Boccalandro, H.E. (2003): Light, phytochrome signalling and photomorphogenesis in *Arabidopsis*. Photochem Photobiol Sci 2 [*6*], 625-636.

Casal, J.J., Fankhauser, C., Coupland, G. and Blázquez, M.A. (2004): Signalling for developmental plasticity. Trends Plant Sci 9 [*6*], 309-314.

Cashmore, A.R., Jarillo, J.A., Wu, Y-J. and Liu, D. (1999): Cryptochromes: Blue Light Receptors for Plants and Animals. Science 284 [*5415*], 760-765.

Chang, C-C., Sheen, J., Bligny, M., Niwa, Y., Lerbs-Mache, S and Stern, D.B. (1999). Functional analysis of two maize cDNAs encoding T7-like RNA polymerases. Plant Cell 11 [*5*], 911–926.

Chattopadhyay, S., Ang, L-H., Puente, P.; Deng, X-W. and Wei, N. (1998): *Arabidopsis* bZIP Protein HY5 Directly Interacts with Light-Responsive Promoters in Mediating Light Control of Gene Expression. Plant Cell 10 [*5*], 673-683.

Chen, M., Chory, J. and Fankhauser, C. (2004): Light Signal Transduction in Higher Plants. Annu Rev Genet 38, 87-117.

Chen, Z., Zheng, Z., Huang, J., Lai, Z. and Fan, B. (2009): Biosynthesis of salicylic acid in plants. Plant Signal Behav 4 [*6*], 493-496.

Chevalier, F., Martin, O., Rofidal, V., Devauchelle, A.D., Barteau, S., Sommerer, N. and Rossignol, M. (2004): Proteomic investigation of natural variation between Arabidopsis ecotypes. Proteomics 4 [*5*], 1372-1381.

Chory, J. (2010): Light signal transduction: an infinite spectrum of possibilities. Plant J 61 [*6*], 982-991.

Chory, J., Reinecke, D., Sim, S., Washburn, T. and Brenner, M. (1994) A role for cytokinins in de-etiolation in *Arabidopsis* (*det* mutants have an altered response to cytokinins). Plant Physiol 104 [*2*], 339-347.

Christie, J.M. (2007): Phototropin Blue-Light Receptors. Annu Rev Plant Biol 58, 21-45.

Christopher, D.A. and Mullet, J.E. (1994): Separate Photosensory Pathways Co-Regulate Blue Light/Ultraviolet-A-Activated *psbD-psbC* Transcription and Light-lnduced D2 and CP43 Degradation in Barley (*Hordeum vulgare*) Chloroplasts. Plant Physiol 104 [*4*], 1119-1129.

Christopher, D.A., Kim, M. and Mullet, J.E. (1992): A novel light-regulated promoter is conserved in cereal and dicot chloroplasts. Plant Cell 4 [*7*], 785–798.

Chun, L., Kawakami, A. and Christopher D.A. (2001): Phytochrome A Mediates Blue Light and UV-A-Dependent Chloroplast Gene Transcription in Green Leaves. Plant Physiol 125 [*4*], 1957-1966.

Courtois, F., Merendino, L., Demarsy, E., Mache, R. and Lerbs-Mache, S. (2007): Phage-type RNA polymerase RPOTmp transcribes the rrn operon from the PC promoter at early developmental stages in Arabidopsis. Plant Physiol 145 [*3*], 712-721.

D'Agostino, I.B. and Kieber, J.J. (1999): Phosphorelay signal transduction: the emerging family of plant response regulators. Trends Biochem Sci 24, 452-456.

Davies, P.J. (2004): Plant Hormones: Biosynthesis, Signal Transduction, Action! (3rd Edition), Kluwer Academic Publishers, Dordrecht, The Netherlands, ISBN: 978-1-4020-2684-3.

Demarsy, E. and Fankhauser, C. (2009): Higher Plants use LOV to perceive blue light. Curr Opin Plant Biol 12 [*1*], 69-74.

Dhingra, A., Bies, D.H., Lehner, K.R. and Folta, K.M. (2006): Green Light Adjusts the Plastid Transcriptome during Early Photomorphogenic Development. Plant Physiol 142 [*3*], 1256-1266.

Du Bell, A.N. and Mullet, J.E. (1995): Differential Transcription of Pea Chloroplast Genes during Light-lnduced Leaf Development. Plant Physiol 109 [*1*], 105-112.

Eisinger, W.R., Bogomolni, R.A. and Taiz, L. (2003): Interactions between a blue-green reversible photoreceptor and a separate UV-B receptor in stomatal guard cells. Am J Bot 90 [*11*], 1560-1566.

Emanuel, C., Weihe, A., Graner, A., Hess, W.R. and Börner, T. (2004): Chloroplast development affects expression of phage-type RNA polymerases in barley leaves. Plant J 38 [*3*], 460-472.

Emanuel, C., von Groll, U., Müller, M., Börner, T. and Weihe, A. (2006): Development- and tissue-specific expression of the *RpoT* gene family of *Arabidopsis* encoding mitochondrial and plastid RNA polymerases. Planta 223 [*5*], 998-1009.

Fluhr, R. and Chua, N.H. (1986): Developmental regulation of two genes encoding ribulose-bisphosphate carboxylase small subunit in pea and transgenic petunia plants: Phytochrome response and blue-light induction. Proc Natl Acad Sci USA 83 [*3*], *2358-2362*.

Folta, K.M. (2004): Green Light Stimulates Early Stem Elongation, Antagonizing Light-Mediated Growth Inhibition. Plant Physiol 135 [*3*], 1407-1416.

Folta, K.M. and Spalding, E.P. (2001): Opposing roles of phytochrome A and phytochrome B in early cryptochrome-mediated growth inhibition. Plant J 28 [*3*], 333-340.

Folta, K.M. and Maruhnich, S.A. (2007): Green light: a signal to slow down or stop. J Exp Bot 58 [*12*], 3099-3111.

Franklin, K.A. and Whitelam, G.C. (2004): Light signals, phytochromes and cross-talk with other environmental cues. J Exp Bot 55 [*395*], 271-276.

Franklin, K.A. and Quail, P.H. (2010): Phytochrome functions in Arabidopsis development. J Exp Bot 61 [*1*], 11-24.

Franklin, K.A., Larner, V.S. and Whitelam, G.C. (2005): The signal transducing photoreceptors of plants. Int J Dev Biol 49 [*5-6*], 653-664.

Galbraith, D.W., Harkins, K.R. and Knapp, S. (1991): Systemic endopolyploidy in *Arabidopsis thaliana*. Plant Physiol. 96 [*3*], 985-989.

Gao, J. and Kaufmann, L.S. (1994): Blue-light Regulation of the *Arabidopsis thaliana Cab1* Gene. Plant Physiol 104 [*4*], 1251-1257.

Gfeller, A., Liechti, R. and Farmer, E.E. (2010): Arabidopsis Jasmonate Signaling Pathway. Sci Signal 3 [*109*], cm4.

Gilroy, S. and Trewavas, A (2001): Signal processing and transduction in plant cells: the end of the beginning? Nat Rev Mol Cell Biol 2 [*4*], 307-314.

Granlund, I., Hall, M., Kieselbach, T. and Schröder, W.P. (2009): Light Induced Changes in Protein Expression and Uniform Regulation of Transcription in the Thylakoid Lumen of *Arabidopsis thaliana*. PLoS ONE 4 [*5*], e5649.

Gray, M.W. (1999): Evolution of organellar genomes. Curr. Opin. Genet. Dev. 9 [*6*], 678-687.

Greenberg, B.M., Gaba, V., Canaani, O., Malkin, S., Mattoo, A.K. and Edelman, M. (1989): Separate photosensitizers mediate degradation of the 32-kDa photosystem II reaction center protein in the visible and UV spectral regions. Proc Natl Acad Sci USA 86 [*17*], 6617-6620.

Guo, H., Yang, H., Mockler, T.C. and Lin, C. (1998): Regulation of flowering time by Arabidopsis photoreceptors. Science 279 [*5355*], 1360-1363.

Haberer, G. and Kieber, J.J. (2002): Cytokinins. New insights into a classic phytohormone. Plant Physio 128 [*2*], 354-362.

Hajdukiewicz, P.T., Allison, L.A. and Maliga, P. (1997): The two RNA polymerases encoded by the nuclear and the plastid compartments transcribe distinct groups of genes in tobacco plastids. EMBO J 16 [*13*], 4041-4048.

Haley, J. and Bogorad, L. (1990): Alternative promoters are used for genes within maize chloroplast polycistronic transcription units. Plant Cell 2 [*4*], 323-333.

Hamazato, F., Shinomura, T., Hanzawa, H., Chory, J. and Furuya, M. (1997): Fluence and wavelength requirements for Arabidopsis *CAB* gene induction by different phytochromes. Plant Physiol 115 [*4*], 1533-1540.

Hande, S. and Jayabaskaran, C. (1996): Cucumber chloroplast *trnL* (CAA) gene - nucleotide sequence and in vivo expression analysis in etiolated cucumber seedlings treated with benzyladenine and light. Indian Journal of Biochemistry & Biophysics 33 [*6*], 448-454.

Hedtke, B., Börner, T. and Weihe, A. (1997): Mitochondrial and chloroplast phage-type RNA polymerases in *Arabidopsis*. Science 277 [*5327*], 809–811.

Hedtke, B., Börner, T. and Weihe, A. (2000): One RNA polymerase serving two genomes. EMBO Rep 1 [*5*], 435–440.

Hedtke, B., Legen, J., Weihe, A., Herrmann, R.G. and Börner, T. (2002) Six active phage-type RNA polymerase genes in *Nicotiana tabacum*. Plant J 30 [*6*], 625–637.

Hertel, S. (2009): Aspekte der plastidären Transkription – der Einfluss des Phytohormons Cytokinin und in vivo-Analysen zur Regulation des rpoB-Operons. PhD thesis, Humboldt-Universität zu Berlin, Mathematisch-Naturwissenschaftliche Fakultät I, urn:nbn:de:kobv:11-100100125.

Hess, W.R. and Börner, T. (1999): Organellar RNA polymerases of higher plants. Int Rev Cytol 190, 1–59.

Heyl, A., Riefler, M., Romanov, G.A. and Schmülling, T. (2011): Properties, functions and evolution of cytokinin receptors. Eur J Cell Biol, doi:10.1016/j.ejcb.2011.02.009 (30.06.11).

Higuchi, M., Pischke, M.S., Mähönen, A.P., Miyawaki, K., Hashimoto, Y., Seki, M., Kobayashi, M., Shinozaki, K., Kato, T., Tabata, S., Helariutta, Y., Sussman, M.R. and Kakimoto, T. (2004): In planta functions of the Arabidopsis cytokinin receptor family. Proc Natl Acad Sci USA 101 [*23*], 8821-8826.

Hirschfeld, M., Tepperman, J.M., Clack, T., Quail, P.H. and Sharrock, R.A. (1998): Coordination of phytochrome levels in *phyB* mutants of *Arabidopsis* as revealed by apoprotein-specific monoclonal antibodies. Genetics 149 [*2*], 523-535.

Hoffer, P.H. and Christopher, D.A. (1997): Structure and Blue-Light-Responsive Transcription of a Chloroplast *psbD* Promoter from *Arabidopsis thaliana*. Plant Physiol 115 [*1*], 213-222.

Hricová, A., Quesada, V. and Micol, J.L. (2006): The SCABRA3 nuclear gene encodes the plastid RpoTp RNA polymerase, which is required for chloroplast biogenesis and mesophyll cell proliferation in Arabidopsis. Plant Physiol 141 [*3*], 942-956.

Huang, M., Abel, C., Sohrabi, R., Petri, J., Haupt, I., Cosimano, J., Gershenzon, J. and Tholl, D. (2010): Variation of Herbivore-Induced Volatile Terpenes among Arabidopsis Ecotypes Depends on Allelic Differences and Subcellular Targeting of Two Terpene Synthases, TPS02 and TPS03. Plant Physiol 153 [*3*], 1293-1310.

Hutchison, C.E. and Kieber, J.J. (2002): Cytokinin signaling in *Arabidopsis*. Plant Cell (Suppl) 14: S47–S59.

Hwang, I. and Sheen, J. (2001): Two-component circuitry in *Arabidopsis* cytokinin signal transduction. Nature 413 [*6854*], 383-389.

Imaizumi, T., Tran, H.G., Swartz, T.E., Briggs, W.R. and Kay, S.A. (2003): FKF1 is essential for photoperiodic-specific light signalling in *Arabidopsis*. Nature 426 [*6964*], 302-306.

Imamura, A., Hanaki, N., Umeda, H. and Nakamura, A. T. S. C. U. T. M. (1998): Response regulators implicated in His-to-Asp phosphotransfer signaling in *Arabidopsis*. Proc Natl Acad Sci USA 95, 2691–2696.

Inoue, S.-I., Kinoshita, T., Matsumoto, M., Nakayama, K.I., Doi, M. and Shimazaki, K.-I. (2008): Blue light-induced autophosphorylation of phototropin is a primary step for signaling. Proc Natl Acad Sci USA 105 [*14*], 5626-5631.

Ishida, K., Yamashino, T., Yokoyama, A. and Mizuno, T. (2009): Three type-B response regulators, ARR1, ARR10 and ARR12, play essential but redundant roles in cytokinin signal transduction throughout the life cycle of Arabidopsis thaliana. Plant Cell Physiol 49 [*1*], 47-57.

Ishizaki, Y., Tsunoyama, Y., Hatano, K., Ando, K., Kato, K., Shinmyo, A., Kobori, M., Takeba, G., Nakahira, Y. and Shiina, T. (2005): A nuclear-encoded sigma factor, Arabidopsis SIG6, recognizes sigma-70 type chloroplast promoters and regulates early chloroplast development in cotyledons. Plant J 42 [*2*], 133-144.

Isono, K., Niwa, Y., Satoh, K. and Kobayashi, H. (1997): Evidence for transcriptional regulation of plastid photosynthesis genes in *Arabidopsis thaliana* roots. Plant Physiol 114 [*2*], 623–630.

Jeong, W.J., Park, Y.I., Suh, K., Raven, J.A., Yoo, O.J. and Liu, J.R. (2002): A large population of small chloroplasts in tobacco leaf cells allows more effective chloroplast movement than a few enlarged chloroplasts. Plant Physiol 129 [*1*], 112-121.

Jiao, Y., Lau, O.S. and Deng, X.W. (2007): Light-regulated transcriptional networks in higher plants. Nat Rev Genet 8 [*3*], 217-230.

Karlin-Neumann, G.A., Sun, L. and Tobin, E.M. (1988): Expression of Light-Harvesting Chlorophyll a/b-Protein Genes Is Phytochrome-Regulated in Etiolated *Arabidopsis thaliana* Seedlings. Plant Physiol 88 [*4*], 1323-1331.

Kasahara, H., Takei, K., Ueda, N., Hishiyama, S., Yamaya, T., Kamiya, Y., Yamaguchi, S. and Sakakibara, H. (2004): Distinct isoprenoid origins of cis- and trans-zeatin biosyntheses in *Arabidopsis*. Journal of Biological Chemistry 279 [*14*], 14049-14054.

Kasten, B., Buck, F., Nuske, J. and Reski, R. (1997): Cytokinin affects nuclear- and plastome-encoded energy converting plastid enzymes. Planta 201 [*3*], 261-272.

Khokhlova, V.A., Karavaiko, N.N., Podergina, T.A. and Kulaeva, O.N. (1978): The antagonistic effect of abscisic acid and cytokinin on the structural and biochemical differentiation of chloroplasts in isolated pumpkin cotyledons. Cytology (Leningrad) 29, 1033-1039.

Kiba, T., Taniguchi, M., Imamura, A., Ueguchi, C., Mizuno, T. and Sugiyama, T. (1999): Differential expression of genes for response regulators in response to cytokinins and nitrate in *Arabidopsis thaliana*. Plant Cell Physiol 40, 767–771.

Kiba, T., Naitou, T., Koizumi, N., Yamashino, T., Sakakibara, H. and Mizuno, T. (2005): Combinatorial microarray analysis revealing *Arabidopsis* genes implicated in cytokinin responses through the His-Asp phosphorelay circuitry. Plant Cell Physiol 46 [*2*], 339–355.

Kim, M. and Mullet, J.E. (1995): Identification of a sequence-specific DNA binding factor required for transcription of the barley chloroplast blue light-responsive *psbD-psbC* promoter. Plant Cell 7 [*9*], 1445–1457.

Kim, M., Thum, K.E., Morishige, D.T. and Mullet, J.E. (1999): Detailed architecture of the barley chloroplast *psbD–psbC* blue light-responsive promoter. J Biol Chem 274 [*8*], 4684–4692.

Kim, W.-Y., Fujiwara, S., Suh, S.-S., Kim, J., Kim, Y., Han, L., David, K., Putterill, J., Nam, H.G. and Somers, D.E. (2007): ZEITLUPE is a circadian photoreceptor stabilized by GIGANTEA in blue light. Nature 449 [*7160*], 356-360.

Klein, R.R. and Mullet, J.E. (1990): Light-induced Transcription of Chloroplast Genes. *psbA* transcription is differentially enhanced in illuminated barley. J Biol Chem 265 [*4*], 1895-1902.

Klein, R.R., Mason, H.S. and Mullet, J.E. (1988): Light-regulated translation of chloroplast proteins. I. Transcripts of *psaA–psaB*, *psbA*, and *rbcL* are associated with polysomes in dark-grown and illuminated barley seedlings. J Cell Biol 106 [*2*], 289–301.

Kleine, T., Lockhart, P. and Batschauer, A. (2003): An Arabidopsis protein closely related to Synechocystis cryptochrome is targeted to organelles. Plant J 35 [*1*], 93-103.

Kobayashi, Y., Dokiya, Y., and Sugita, M. (2001): Dual targeting of phage-type RNA polymerase to both mitochondria and plastids is due to alternative translation initiation in single transcripts. Biochem Biophys Res Commun 289 [*5*], 1106-1113.

Koiwai, H., Nakaminami, K., Seo, M., Mitsuhashi, W., Toyomasu, T. and Koshiba, T. (2004): Tissue-specific localization of an abscisic acid biosynthetic enzyme, AOO3, in *Arabidopsis*. Plant Physiol 134 [*4*], 1697-1707.

Koornneef, M., Rolff, E. and Spruit, C.J.P. (1980). Genetic control of light-inhibited hypocotyl elongation in *Arabidopsis thaliana* (L.) Heynh. Zeitschrift für Pflanzenphysiologie 100, 147-160.

Koornneef, M., Hanhart, C.J. and van der Veen, J.H. (1991): A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. Mol Gen Genet 229 [*1*], 57-66.

Kühn, K., Richter, U., Meyer, E.H., Delannoy, E., de Longevialle, A.F., O'Toole, N., Börner, T., Millar, A.H., Small, I.D. and Whelan, J. (2009): Phage-type RNA polymerase RPOTmp performs gene-specific transcription in mitochondria of *Arabidopsis thaliana*. Plant Cell 21 [*9*], 2762-2779.

Kulaeva, O.N., Karavaiko, N.N., Selivankina, S.Y., Kusnetsov, V.V., Zemlyachenko, Y.V., Cherepneva, G.N., Maslova, G.G., Lyukevich, T.V., Smith, A.R. and Hall, M.A. (2000): Nuclear and chloroplast cytokinin-binding proteins from barley leaves participating in transcription regulation. Plant Growth Regulation 32 [*2*], 329-335.

Kurakawa, T., Ueda, N., Maekawa, M., Kobayashi, K., Kojima, M., Nagato, Y., Sakakibara, H. and Kyozuka, J. (2007): Direct control of shoot meristem activity by a cytokinin-activating enzyme. Nature 445 [*7128*], 652-655.

Kusnetsov, V.V., Oelmüller, R., Sarwat, M.I., Porfirova, S.A., Cherepneva, G.N., Herrmann, R.G. and Kulaeva, O.N. (1994): Cytokinins, abscisic acid and light affect accumulation of chloroplast proteins in *Lupinus luteus* cotyledons without notable effect on steady-state mRNA levels. Planta 194, 318-327.

Kusumi, K., Yara, A., Mitsui, N., Tozawa, Y. and Iba, K. (2004): Characterization of a rice nuclear-encoded plastid RNA polymerase gene *OsRpoTp*. Plant Cell Physiol 45 [*9*], 1194–1201.

Larkin, R.M. and Ruckle, M.E. (2008): Integration of light and plastid signals. Curr Opin Plant Biol 11 [*6*], 593-599.

Lau, O.S. and Deng, X.W. (2010): Plant hormone signaling lightens up: integrators of light and hormones. Curr Opin Plant Biol 13 [*5*], 571-577.

Lee, J., He, K., Stolc, V., Lee, H., Figueroa, P., Gao, Y., Tongprasit, W., Zhao, H., Lee, I. and Deng, X.W. (2007): Analysis of Transcription Factor HY5 Genomic Binding Sites Revealed Its Hierarchical Role in Light Regulation of Development. Plant Cell 19 [*3*], 731-749.

Lerbs, S., Lerbs, W., Klyachko, N. L., Romanko, E. G., Wollgiehn, R. and Parthier, B. (1984): Gene expression in cytokinin-and light-mediated plastogenesis of *Cucurbita cotyledons*: ribulose-1,5-bisphosphate carboxylase/oxygenase. Planta 162 [*4*], 289-298.

Lerbs-Mache, S. (2011): Function of plastid sigma factors in higher plants: regulation of gene expression or just preservation of constitutive transcription? Plant Mol Biol 76 [*3-5*], 235-249.

Li, Q-H. and Yang, H-Q. (2007): Cryptochrome Signaling in Plants. Photochem Photobiol 83 [*1*], 94-101.

Li, J., Li, G., Gao, S., Martinez, C., He, G., Zhou, Z., Huang, X., Lee, J.H., Zhang, H., Shen, Y., Wang, H. and Deng, X.W. (2010): *Arabidopsis* Transcription Factor ELONGATED HYPOCOTYL5 Plays a Role in the Feedback Regulation of Phytochrome A Signaling. Plant Cell 22 [*11*], 3634-3649.

Li, W., Ruf, S. and Bock, R. (2006): Constancy of organellar genome copy numbers during leaf development and senescence in higher plants. Mol Genet Genomics 275 [*2*], 185-192.

Liere K. and Börner, T. (2007a): Transcription of plastid genes. In: Grasser, K.D., editor. Regulation of transcription in plants. Oxford: Blackwell Publishing, 184-224.

Liere, K. and Börner, T. (2007b): Transcription and transcriptional regulation in plastids. In: Bock, R., editor. Topics in current genetics: cell and molecular biology of plastids. Berlin/Heidelberg: Springer, 121-174.

Liere, K., Weihe, A. and Börner, T. (2011): The transcription machineries of plant mitochondria and chloroplasts: Composition, function, and regulation. J Plant Physiol 168 [*15*], 1345-1360.

Liere, K., Kaden, D., Maliga, P. and Börner, T. (2004): Overexpression of phage-type RNA polymerase RpoTp in tobacco demonstrates its role in chloroplast transcription by recognizing a distinct promoter type. Nucleic Acids Res 32 [*3*], 1159-1165.

Lin, C. (2002): Blue Light Receptors and Signal Transduction. Plant Cell 14, S207-225.

Lin, C. and Shalitin, D. (2003): Cryptochrome Structure and Signal Transduction. Annual Review of Plant Biology 54, 469-496.

Lin, C. and Todo, T. (2005): The cryptochromes. Genome Biol 6 [*5*], 220.1-220.9.

Lin, Z., Zhong, S. and Grierson, D. (2009): Recent advances in ethylene research. J Exp Bot 60 [*12*], 3311-3336.

Link, G. (1996): Green life: control of chloroplast gene transcription. Bio Essays 18 [*6*], 465-471.

Loschelder, H., Schweer, J., Link, B. and Link, G. (2006): Dual temporal role of plastid sigma factor 6 in Arabidopsis development. Plant Physiol 142 [*2*], 642-650.

Lysenko, EA (2007): Plant sigma factors and their role in plastid transcription. Plant Cell Rep 26, 845-859.

Lyukevich, T.V., Kusnetsov, V.V., Karavaiko, N.N., Kulaeva, O.N. and Selivankina, S.Y. (2002): The involvement of the chloroplast zeatin-binding protein in hormone-dependent transcriptional control of the chloroplast genome. Rus J Plant Physiol 49, 92–98.

Ma, L., Li, J., Qu, L., Hager, J., Chen, Z., Zhao, H. and Deng, X.W. (2001): Light control of Arabidopsis development entails coordinated regulation of genome expression and cellular pathways. Plant Cell 13 [*12*], 2589-2607.

Maliga, P. (1998): Two plastid polymerases of higher plants: an evolving story. Trends Plant Sci 3, 4-6.

Mandoli, D.F. and Briggs, W.R. (1981): Phytochrome control of two low-irradiance responses in etiolated oat seedlings. Plant Physiol 67 [*4*], 733-739.

Martin, W., Hoffmeister, M., Rotte, C. and Henze, K. (2001): An overview of endosymbiotic models for the origins of eukaryotes, their ATP-producing organelles (mitochondria and hydrogenosomes), and their heterotrophic lifestyle. Biol Chem 382 [*11*], 1521-1539.

Martin, W., Rujan, T., Richly, E., Hansen, A., Cornelsen, S., Lins, T., Leister, D., Stoebe, B., Hasegawa, M. and Penny, D. (2002) Evolutionary analysis of *Arabidopsis*, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. Proc Natl Acad Sci USA 99 [*19*], 12246–12251.

Más, P., Devlin, P.F., Panda, S. and Kay, S.A. (2000): Functional interaction of phytochrome B and cryptochrome 2. Nature 408 [*6809*], 207-211.

Mason, M.G, Li, J., Mathews, D.E., Kieber, J.J. and Schaller, G.E. (2004): Type-B response regulators display overlapping expression patterns in *Arabidopsis*. Plant Physiol 135 [*2*], 927-937.

Masuda, T., Tanaka, R., Shioi, Y., Takamiya, K.-I., Kannangara, C.G. and Tsuji, H. (1994): Mechanism of benzyladenine-induced stimulation of the synthesis of 5-aminolevulinic acid in greening cucumber cotyledons: benzyladenine increases levels of plastid tRNAGlu. Plant Cell Physiol 35 [*2*], 183-188.

Mathews, S. and Sharrock, R.A. (1997): Phytochrome gene diversity. Plant Cell Environ 20 [*6*], 666–671.

Mayfield, S.P., Yohn, C.B., Cohen, A. and Danon, A. (1995): Regulation of Chloroplast Gene Transcription. Annu Rev Plant Physiol Plant Mol Biol 46, 147-166.

Mazzella, M.A., Cerdan, P.D., Staneloni, R.J. and Casal, J.J. (2001): Hierarchical coupling of phytochromes and cryptochromes reconciles stability and light modulation of Arabidopsis development. Development 128 [*12*], 2291-2299.

Miller, C.O., Skoog, F., von Saltza, M.H. and Strong, F.M. (1955): Kinetin, a cell division factor from deoxyribonucleic acid. J Am Chem Soc 77, 1392-1393.

Miller, C.O., Skoog, F., Okomura, F., von Saltza, M.H. and Strong, F.M. (1956): Isolation, structure and synthesis of kinetin, a substance promoting cell division. J Am Chem Soc 78, 1375-1380.

Mochizuki, T., Onda, Y., Fujiwara, E., Wada, M. and Toyoshima, Y. (2004): Two independent light signals cooperate in the activation of the plastid *psbD* blue light-responsive promoter in Arabidopsis. FEBS Lett 571 [*1-3*] 26-30.

Mockler, T.C., Guo, H., Yang, H., Duong, H. and Lin, C. (1999): Antagonistic actions of Arabidopsis cryptochromes and phytochrome B in the regulation of floral induction. Development 126 [*10*], 2073-2082.

Mok, D.W.S. and Mok, M.C. (2001): Cytokinin Metabolism and Action. Annu Rev Plant Physiol Plant Mol Biol 52, 89-118.

Monde, R.A., Schuster, G. and Stern, D.B. (2000): Processing and degradation of chloroplast mRNA. Biochimie 82 [*6-7*], 573–582.

Mullet, J.E. (1993): Dynamic Regulation of Chloroplast Transcription. Plant Physiol 103 [*2*], 309-313.

Mullet, J.E. and Klein, R.R. (1987): Transcription and RNA stability are important determinants of higher plant chloroplast RNA levels. EMBO J 6 [*6*], 1571-1579.

Nagashima, A., Hanaoka, M., Shikanai, T., Fujiwara, M., Kanamaru, K., Takahashi, H. and Tanaka, K. (2004): The multiple-stress responsive plastid sigma factor, SIG5, directs activation of the *psbD* blue light-responsive promoter (BLRP) in *Arabidopsis thaliana*. Plant Cell Physiol 45 [*4*], 357–368.

Nagatani, A., Reed, J.W. and Chory, J. (1993): Isolation and Initial Characterization of Arabidopsis Mutants That Are Deficient in Phytochrome A. Plant Physiol 102 [*1*], 269-277.

Nakamura, T., Furuhashi, Y., Hasegawa, K., Hashimoto, H., Watanabe, K., Obokata, J., Sugita, M. and Sugiura, M. (2003): Array-Based Analysis on Tobacco Plastid Transcripts: Preparation of a Genomic Microarray Containing All Genes and All Intergenic Regions. Plant Cell Physiol 44 [*8*], 861-867.

Neff, M.M. and Chory, J. (1998): Genetic Interactions between Phytochrome A, Phytochrome B, and Cryptochrome 1 during Arabidopsis Development. Plant Physiol 118 [*1*], 27-35.

Nishimura, C., Ohashi, Y., Sato, S., Kato, T., Tabata, S. and Ueguchi, C. (2004): Histidine kinase homologs that act as cytokinin receptors possess overlapping functions in the regulation of shoot and root growth in Arabidopsis. Plant Cell 16 [*6*], 1365-1377.

Ohgishi, M., Saji, K., Okada, K. and Sakai, T. (2004): Functional analysis of each blue light receptor, cry1, cry2, phot1, and phot2, by using combinatorial multiple mutants in *Arabidopsis*. Proc Natl Acad Sci USA 101 [*8*], 2223-2228.

Okazaki, K., Kabeya, Y., Suzuki, K., Mori, T., Ichikawa, T., Matsui, M., Nakanishi, H. and Miyagishima, S.Y. (2009): The PLASTID DIVISION1 and 2 Components of the Chloroplast Division Machinery Determine the Rate of Chloroplast Division in Land Plant Cell Differentiation. Plant Cell 21 [*6*], 1769-1780.

Oldenburg, D.J. and Bendich, A.J. (2004): Changes in the structure of DNA molecules and the amount of DNA per plastid during chloroplast development in maize. J Mol Biol 344 [*5*], 1311-1330.

Onda, Y., Yagi, Y., Saito, Y., Takenaka, N. and Toyoshima, Y. (2008): Light induction of *Arabidopsis SIG1* and *SIG5* transcripts in mature leaves: differential roles of cryptochrome 1 and cryptochrome 2 and dual function of SIG5 in the recognition of plastid promoters. Plant J 55 [*6*], 968–978.

Osterlund, M.T., Hardtke, C.S., Wei, N. and Deng X-W. (2000): Targeted destabilization of HY5 during light-regulated development of *Arabidopsis*. Nature 405 [*6785*], 462-466.

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

Peng, Z.Y., Zhou, X., Li, L., Yu, X., Li, H., Jiang, Z., Cao, G., Bai, M., Wang, X., Jiang, C., Lu, H., Hou, X., Qu, L., Wang, Z., Zuo, J., Fu, X., Su, Z., Li, S. and Guo, H. (2009): Arabidopsis

Hormone Database: a comprehensive genetic and phenotypic information database for plant hormone research in *Arabidopsis*. Nucleic Acid Res 37, D975-982.

Peschke, F. and Kretsch, T. (2011): Genome-wide analysis of light-dependent transcript accumulation patterns during early stages of Arabidopsis seedling deetiolation. Plant Physiol 155 [*3*], 1353-1366.

Pfannschmidt, T. and Liere, K. (2005). Redox regulation and modification of proteins controlling chloroplast gene expression. Antioxid. Redox. Signal. 7 [*5-6*], 607-618.

Pfannschmidt, T., Nilsson, A. and Allen, J.F. (1999a): Photosynthetic control of chloroplast gene expression. Nature 397 [*6720*], 625-628.

Pfannschmidt, T., Nilsson, A., Tullberg, A., Link, G. and Allen, J.F. (1999b): Direct transcriptional control of the chloroplast genes *psbA* and *psaAB* adjusts photosynthesis to light energy distribution in plants. IUBMB Life 48 [*3*], 271-276.

Polanská, L., Vicánková, A., Nováková, M., Malbeck, J., Dobrev, P., Brzobohaty, B., Vanková, R. and Macháckova, I. (2007): Altered cytokinin metabolism affects cytokinin, auxin, and abscisic acid contents in leaves and chloroplasts, and chloroplast ultrastructure in transgenic tobacco. J Exp Bot 58 [*3*], 637-649.

Poppe, C., Sweere U., Drumm-Herrel, H. and Schäfer, E. (1998): The blue light receptor cryptochrome 1 can act independently of phytochrome A and B in *Arabidopsis thaliana*. Plant J 16 [*4*], 465-471.

Preuten, T. (2010): Organellar gene expression – regulation of phage-type RNA polymerase transcript accumulation and analyses of mitochondrial gene copy numbers in Arabidopsis. PhD thesis, Humboldt-Universität zu Berlin, Mathematisch-Naturwissenschaftliche Fakultät I, urn:nbn:de:kobv:11-100173056.

Quail, P.H. (2002): Phytochrome photosensory signalling networks. Nat Rev Mol Cell Biol 3 [*2*], 85-93.

Quail, P.H. (2010): Phytochromes. Curr Biol 20[*12*], R504-507.

Quail, P.H., Boylan, M.T., Parks, B.M., Short, T.W., Xu, Y. and Wagner, D. (1995): Phytochromes: Photosensory Perception and Signal Transduction. Science 268 [*5211*], 675-680.

Rapp, J.C., Baumgartner, B.J. and Mullet, J.E. (1992): Quantitative Analysis of Transcription and RNA Levels of 15 Barley Chloroplast Genes. Transcription rates and mRNA levels vary over 300-fold; predicted mRNA stabilities vary 30-fold. J Biol Chem 267 [*30*], 21404-21411.

Rashotte, A.M. and Goertzen, L.R. (2010): The CRF domain defines cytokinin response factor proteins in plants. BMC Plant Biol 10 [*74*], doi: 10.1186/1471-2229-10-74.

Rashotte, A.M., Carson, S.D., To, J.P. and Kieber, J.J. (2003): Expression profiling of cytokinin action in *Arabidopsis*. Plant Physiol 132 [*4*], 1998-2011.

Rashotte, A.M., Mason, M.G., Hutchison, C.E., Ferreira, F.J., Schaller, G.E. and Kieber, J.J. (2006): A subset of *Arabidopsis* AP2 transcription factors mediates cytokinin responses in concert with a two-component pathway. Proc Natl Acad Sci USA 103 [*29*], 11081-11085.

Rauwolf, U., Golczyk, H., Greiner, S. and Herrmann, R.G. (2010): Variable amounts of DNA related to the size of chloroplasts III. Biochemical determinations of DNA amounts per organelle. Mol Genet Genomics 283 [*1*], 35-47.

Razem, F.A., Baron, K. and Hill, R.D. (2006): Turning on gibberellin and abscisic acid signaling. Curr Opin Plant Biol 9 [*5*], 454-459.

Reed, J.W., Nagatani, A., Elich, T.D., Fagan, M. and Chory, J. (1994): Phytochrome A and phytochrome B have overlapping but distinct functions in Arabidopsis development. Plant Physiol 104 [*4*], 1139-1149.

Richmond, A.E. and Lang, A. (1957): Effect of Kinetin on Protein Content and Survival of Detached Xanthium Leaves. Science 125 [*3249*], 650-651.

Richter, U., Kiessling, J., Hedtke, B., Decker, E., Reski, R., Börner, T. and Weihe, A. (2002): Two *RpoT* genes of *Physcomitrella patens* encode phage-type RNA polymerases with dual targeting to mitochondria and plastids. Gene 290 [*1-2*], 95–105.

Riefler, M., Novak, O., Strnad, M. and Schmülling, T. (2006): *Arabidopsis* cytokinin receptor mutants reveal functions in shoot growth, leaf senescence, seed size, germination, root development, and cytokinin metabolism. Plant Cell 18 [*1*], 40-54.

Rivero, R.M., Kojima, M., Gepstein, A., Sakakibara, H., Mittler, R., Gepstein, S. and Blumwald, E. (2007): Delayed leaf senescence induces extreme drought tolerance in a flowering plant. Proc Natl Acad Sci USA 104 [*49*], 19631-19636.

Rogers, S.O., Beaulieu, G.C. and Bendich, A.J. (1993): Comparative studies of gene copy number. Methods in Enzymology 224, 243-251.

Romanko, E.G., Hein, H.J., Kulaeva, O.N. and Nichiporovich, A.A. (1969): Effect of cytokinin on the physiological activity of chloroplasts. Progress Photosynthesis Research 1: 296-303.

Ruckle, M.E., De Marco, S.M. and Larkin, R.M. (2007): Plastid signals remodel light signaling networks and are essential for efficient chloroplast biogenesis in *Arabidopsis*. Plant Cell 19 [*12*], 3944-3960.

Sakai, H., Aoyama, T. and Oka, A. (2000): Arabidopsis ARR1 and ARR2 response regulators operate as transcriptional activators. Plant J 24 [*6*], 703-711.

Sakakibara, H. (2006): Cytokinins: activity, biosynthesis, and translocation. Annu Rev Plant Biol 57, 431-449.

Sambrook, J. and Russell, D. W. (2001): Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; ISBN: 0879695773.

Santner, A., Calderon-Villalobos L.I. and Estelle, M. (2009): Plant hormones are versatile chemical regulators of plant growth. Nat Chem Biol 5 [*5*], 301-307.

Satoh, J., Baba, K., Nakahira, Y., Shiina, T. and Toyoshima, Y. (1997): Characterization of dynamics of the *psbD* light-induced transcription in mature wheat chloroplasts. Plant Mol Biol 33 [*2*], 267-278.

Schafer, E. and Haupt, W. (1983): Blue light effects in phytochrome-mediated responses. In Encyclopedia of Plant Physiology, Shropshire Jr., W. and Mohr, H. eds (New York: Springer-Verlag).

Schmülling, T. (2004): Cytokinin. In Encyclopedia of Biological Chemistry (Eds. Lennarz, W., Lane, M.D.), Academic Press/Elsevier Science.

Schrubar, H., Wanner, G. and Westhoff, P. (1990): Transcriptional control of plastid gene expression in greening Sorghum seedlings. Planta 183, 101-111.

Schweer, J. (2010): Plant sigma factors come of age: flexible transcription factor network for regulated plastid gene expression. Endocytobio Cell Res 20, 1–20.

Sellaro, R., Hoecker, U., Yanovsky, M., Chory, J. and Casal, J.J. (2009): Synergistic interactions of red and blue light in the control of Arabidopsis gene expression and development. Curr Biol 19 [*14*], 1216-1220.

Serino, G. and Maliga, P. (1998): RNA polymerase subunits encoded by the plastid rpo genes are not shared with the nucleus-encoded plastid enzyme. Plant Physiol 117 [*4*], 1165–1170.

Sexton, T.B., Christopher, D.A. and Mullet, J.E. (1990): Light-induced switch in barley *psbD-psbC* promoter utilization: a novel mechanism regulating chloroplast gene expression. EMBO J 9 [*13*], 4485-4494.

Shiina, T., Allison, L. and Maliga, P. (1998): *rbcL* Transcript Levels in Tobacco Plastids Are Independent of Light: Reduced Dark Transcription Rate Is Compensated by Increased mRNA Stability. Plant Cell 10 [*10*], 1713-1722.

Shiina, T., Tsunoyama, Y., Nakahira, Y., Khan, M.S. (2005): Plastid RNA polymerases, promoters, and transcription regulators in higher plants. Int Rev Cytol 244, 1–68.

Shimizu, M., Kato, H., Ogawa, T., Kurachi, A., Nakagawa, Y. and Kobayashi, H. (2010): Sigma factor phosphorylation in the photosynthetic control of photosystem stoichiometry. Proc Natl Acad Sci USA 107 [*23*], 10760-10764.

Shinomura, T., Nagatani, A., Hanzawa, H., Kubota, M., Watanabe, M. and Furuya, M. (1996): Action spectra for phytochrome A- and B-specific photoinduction of seed germination in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 93 [*15*], 8129-8133.

Shirano, Y., Shimada, H., Kanamaru, K., Fujiwara, M., Tanaka, K., Takahashi, H., Unno, K., Sato, S., Tabata, S., Hayashi, H., Miyake, C., Yokota, A. and Shibata, D. (2000): Chloroplast development in *Arabidopsis thaliana* requires the nuclear-encoded transcription factor sigma B. FEBS Lett 485 [*2-3*], 178-182.

Skoog, F. and Miller, C.O. (1957): Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp Soc Exp Biol 11, 118-131.

Smith, H. (2000): Phytochromes and light signal perception by plants—an emerging synthesis. Nature 407 [*6804*], 585-591.

Spalding, E.P. and Folta, K.M. (2005): Illuminating topics in plant photobiology. Plant Cell Environ 1, 39-51.

Stabel, P., Sundås, A. and Engström, P. (1991): Cytokinin treatment of embryos inhibits the synthesis of chloroplast proteins in Norway spruce. Planta 183 [*4*], 520-527.

Stegemann, S., Hartmann, S., Ruf, S. and Boch, R. (2003): High-frequency gene transfer from the chloroplast genome to the nucleus. Proc Natl Acad Sci USA 100 [*15*], 8828-8833.

Stern, D.B., Goldschmidt-Clermont, M. and Hanson, M.R. (2010): Chloroplast RNA metabolism. Annu Rev Plant Biol 61, 125–155.

Strasser, B., Sánchez-Lamas, M., Yanovsky, M.J., Casal, J.J. and Cerdán, P.D. (2010): *Arabidopsis thaliana* life without phytochromes. Proc Natl Acad Sci USA 107 [*10*] 4776-4781.

Strnad, M. (1997): The aromatic cytokinins. Physiol Plant 101 [*4*], 674-688.

Sugiura, M. (1992): The chloroplast genome. Plant Mol Biol 19 [*1*], 149-168.

Suzuki, T., Ishikawa, K. and Mizuno, T. (2002): An Arabidopsis histidine-containing phosphotransfer (Hpt) factor implicated in phosphorelay signal transduction: Overexpression of AHP2 in plants results in hypersensitivity to cytokinin. Plant Cell Physiol 43 [*1*], 123-129.

Suzuki, J.Y., Ytterberg, A.J., Beardslee, T.A., Allison, L.A., Wijk, K.J. and Maliga, P. (2004): Affinity purification of the tobacco plastid RNA polymerase and in vitro reconstitution of the holoenzyme. Plant J 40 [*1*], 164–172.

Swiatecka-Hagenbruch, M., Liere, K. and Börner, T. (2007): High diversity of plastidial promoters in *Arabidopsis thaliana*. Mol Genet Genomics 277 [*6*], 725-734.

Swiatecka-Hagenbruch, M., Emanuel, C., Hedtke, B., Liere, K. and Börner, T. (2008): Impaired function of the phage-type RNA polymerase RpoTp in transcription of chloroplast genes is compensated by a second phage-type RNA polymerase. Nucleic Acids Res. 36 [*3*], 785-792.

Székács, A., Hegedüs, G., Tóbiás, I., Pogány, M. and Barna, B. (2000): Immunoassays for plant cytokinins as tools for the assessment of environmental stress and disease resistance. Analytica Chimica Acta 421 [*2*], 135-146.

Takagi, S., Takamatsu, H. and Sakurai-Ozato, N. (2009): Chloroplast anchoring: its implications for the regulation of intracellular chloroplast distribution. J Exp Bot 60 [*12*], 3301-3310.

Tepperman, J.M., Hwang, Y-S. and Quail P.H. (2006): phyA dominates in transduction of red-light signals to rapidly responding genes at the initiation of Arabidopsis seedling de-etiolation. Plant J 48 [*5*], 728-742.

Tepperman, J.M., Zhu, T., Chang, H-S., Wang, X. and Quail, P.H. (2001): Multiple transcription-factor genes are early targets of phytochrome A signaling. Proc Natl Acad Sci USA 98 [*16*], 9437-9442.

Tepperman, J.M., Hudson, M.E., Khanna, R., Zhu, T., Chang, S.H., Wang, X. and Quail, P.H. (2004): Expression profiling of phyB mutant demonstrates substantial contribution of other phytochromes to red-light-regulated gene expression during seedling de-etiolation. Plant J 38 [*5*], 725-739.

Terzaghi, W.B. and Cashmore, A.R. (1995): Photomorphogenesis. Seeing the light in plant development. Curr Biol 5 [*5*], 466-468.

Thompson, W.F. and White, M.J. (1991): Physiological and molecular studies of light-regulated nuclear genes in higher plants. Plant Physiol Plant Mol Biol 42, 423-466.

Thum, K.E., Kim, M., Christopher, D.A. and Mullet, J.E. (2001): Cryptochrome 1, Cryptochrome 2, and Phytochrome A Co-Activate the Chloroplast *psbD* Blue Light–Responsive Promoter. Plant Cell 13 [*12*], 2747-2760.

Timmis, J.N., Ayliffe, M.A., Huang, C.Y. and Martin, W. (2004): Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. Nat Rev Genet 5 [*2*], 123–135.

To, J.P.C. and Kieber, J.J. (2008): Cytokinin signaling: two-components and more. Trends Plant Sci 13 [*2*], 85-92.

To, K.Y., Cheng, M.C., Suen, D.F., Mon, D.P., Chen, L.F.O. and Chen, S.C.G. (1996): Characterization of the light-responsive promoter of rice chloroplast psbD-C operon and the sequence-specific DNA binding factor. Plant Cell Physiol 37 [*5*], 660–666.

Toyoshima, Y., Onda, Y., Shiina, T. and Nakahira, Y. (2005): Plastid transcription in higher plants. Crit. Rev. Plant Sci. 24 [*1*], 59–81.

Tsuchida-Mayama, T., Sakai, T., Hanada, A., Uehara, Y., Asami, T. and Yamaguchi, S. (2010): Role of the phytochrome and cryptochrome signaling pathways in hypocotyl phototropism. Plant J. 62 [*4*], 653-662.

Tsunoyama, Y., Morikawa, K., Shiina, T. and Toyoshima, Y. (2002): Blue light specific and differential expression of a plastid sigma factor, Sig5 in *Arabidopsis thaliana*. FEBS Lett 515 [*1-3*], 225-228.

Tsunoyama, Y., Ishizaki, Y., Morikawa, K., Kobori, M., Nakahira, Y., Takeba, G., Toyoshima, Y. and Shiina, T. (2004): Blue light-induced transcription of plastid-encoded *psbD* gene is mediated by a nuclear-encoded transcription initiation factor, *AtSig5*. Proc Natl Acad Sci USA 101 [*9*], 3304-3309.

Tyagi, A.K. and Gaur, T. (2003): Light regulation of nuclear photosynthetic genes in higher plants. Crit Rev Plant Sci 22 [*5*], 417-452.

Ulm, R. and Nagy, F. (2005): Signalling and gene regulation in response to ultraviolet light. Curr Opin Plant Biol 8 [*5*], 477-482.

Ulm, R., Baumann, A., Oravecz, A., Máté, Z., Adám, E., Oakeley, E.J., Schäfer, E. and Nagy, F. (2004): Genome-wide analysis of gene expression reveals function of the bZIP transcription factor HY5 in the UV-B response of *Arabidopsis*. Proc Natl Acad Sci USA 101 [*5*], 1397-1402.

Vandenbussche, F., Habricot, Y., Condiff, A.S., Maldiney, R., Van Der Straeten, D. and Ahmad, M. (2007): HY5 is a point of convergence between cryptochrome and cytokinin signaling pathways in *Arabidopsis thaliana*. Plant J 49 [*3*], 428-441.

Wada, T., Tunoyama, Y., Shiina, T. and Toyoshima, Y. (1994): In vitro analysis of light-induced transcription in the wheat *psbD/C* gene cluster using plastid extracts from dark-grown and short-term-illuminated seedlings. Plant Physiol 104 [*4*], 1259–1267.

Wakasugi, T., Tsudzuki, T. and Sugiura, M. (2001): The genomics of land plant chloroplasts: gene content and alteration of genomic information by RNA editing. Photosynthesis Res 70 [*1*], 107-118.

Weiler, E.W. (2003) Sensory principles of higher plants: Plant Biochemistry. In: Angew Chem Int Ed. ISSN: 1521-3773; Wiley-VCH, Weinheim, 392-411.

Werner, T. and Schmülling, T. (2009): Cytokinin action in plant development. Curr Opin Plant Biol 12 [*5*], 527-538.

Werner, T., Motyka, V., Laucou, V., Smets, R., Van Onckelen, H. and Schmülling, T. (2003): Cytokinin-deficient transgenic *Arabidopsis* plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. Plant Cell 15 [*11*], 2532-2550.

Wösten, M.M.S.M. (1998): Eubacterial sigma-factors. FEMS Microbiol Rev 22 [*3*], 127-150.

Woodson, J.D. and Chory, J. (2008): Coordination of gene expression between organellar and nuclear genomes. Nat Rev Genet 9 [*5*], 383-395.

Wu, G. and Spalding, E.P. (2007): Separate functions for nuclear and cytoplasmic cryptochrome 1 during photomorphogenesis of *Arabidopsis* seedlings. Proc Natl Acad Sci USA 104 [*47*], 18813-18818.

Yoshida, K. and Noguchi, K. (2011): How is the Respiratory Gene Expression Regulated in Illuminated Leaves? In: Kempken, F., editor. Advances in Plant Biology 1: Plant Mitochondria. Berlin/Heidelberg: Springer, 396-410.

Yu, X., Liu, H., Klejnot, J. and Lin, C. (2010): The Cryptochrome Blue Light Receptors. In: The American Society of Plant Biologists. The Arabidopsis Book 8 [*1*], 1-27, URL: http://www.bioone.org/doi/full/10.1043/tab.00135 (23.01.11).

Zhao, Y. (2010): Auxin biosynthesis and its role in plant development. Annu Rev Plant Biol 61, 49-64.

Zhang, X.N., Wu, Y., Tobias, J.W., Brunk, B.P., Deitzer, G.F. and Liu, D. (2008): HFR1 is crucial for transcriptome regulation in the cryptochrome 1-mediated early response to blue light in *Arabidopsis thaliana*. PLoS One 2 [*10*], e3563.

Zheng, B., Deng, Y., Mu, J., Ji, Z., Xiang, T., Niu, Q-W., Nhua, N.-H. and Zuo, J. (2006): Cytokinin affects circadian-clock oscillation in a phytochrome B- and Arabidopsis response regulator 4-dependent manner. Physiologia Plantarum 127 [*2*], 277-292.

Zheng, Q, Oldenburg, D.J. and Bendich, A.J. (2011): Independent effects of leaf growth and light on the development of the plastid and its DNA content in Zea species. J Exp Bot 62 [*8*], 2715-2730.

Zoschke, R., Liere, K. and Börner, T. (2007): From seedling to mature plant: Arabidopsis plastidial genome copy number, RNA accumulation and transcription are differentially regulated during leaf development. Plant J 50 [*4*], 710-722.

Zubo, Y.O. and Kusnetsov, V.V. (2008): Application of Run-on Transcription Method for Studying the Regulation of Plastid Genome Expression. Russian Journal of Plant Physiology 55 [*1*], 107-114.

Zubo, Y.O., Yamburenko, M.V., Selivankina, S.Y., Shakirova, F.M., Avalbaev, A.M., Kudryakova, N.V., Zubkova, N.K., Liere, K., Kulaeva, O.N., Kusnetsov, V.V. and Börner, T. (2008): Cytokinin stimulates chloroplast transcription in detached barley leaves. Plant Physiol 148 [*2*], 1082-1093.

Abbreviations

°C	degrees Celsius
A, C, G, T, U	nucleic acid bases (adenine, cytosine, guanine, thymin, uracil)
ABA	abscisic acid
AHK	Arabidopsis Histidine Kinase
AHP	Arabidopsis Histidine Phosphotransfer protein
ARR	Arabidopsis Response Regulator
A.th.	*Arabidopsis thaliana*
ATP	adenosine triphosphate
b	blue light
BA	6-benzyladenine (cytokinin)
BLRP	blue light responsive promoter
BR	brassinosteroid
BSA	bovine serum albumin
bp	base pairs
C	ploidy number
cDNA	complementary DNA
Ci	Curie
CKX	cytokinin oxidase/dehydrogenase
cm	centimeter
Col-0	ecotype Columbia (*Arabidopsis thaliana*)
COP	constitutive photomorphogenic protein
CRF	Cytokinin Response Factors
cry	cryptochrome
CTP	cytidine triphosphate
d	darkness or day(s)
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
e.g.	*exempli gratia* (for example)
et al.	*et altera* (and others)
EtBr	ethidium bromide
fr	far-red light
g	gram or gravity or green light
GA	gibberellic acid
GABI-Kat	german plant genomics research program
gDNA	genomic DNA
GTP	guanosine triphosphate
h	hour(s)
HCl	hydrochloric acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
hy	long hypocotyl
IAA	indole-3-acetic acid or auxin
i.e.	*id est* (that is)
IgG	Immunoglobulin G
JA	jasmonic acid
kDa	kilodalton
KOH	potassium hydroxide
kPa	kilopascal
l	liter
LED	light emitting diode
L*er*	ecotype Landsberg *erecta* (*Arabidopsis thaliana*)
m	meter
M	molar

mg	milligram
µg	microgram
Mg_2Cl	magnesium chloride
mm	millimeter
µm	micrometer
µM	micromolar
µmol	micromol
min	minute(s)
n	number of nuclei
NaAc	sodium acetate
NaCl	sodium chloride
Na_2HPO_4	disodium hydrogen phosphate
NaOH	sodium hydroxide
NASC	Nottingham *Arabidopsis* Stock Center
NEP	nuclear encoded plastid RNA polymerase
nm	nanometer
NTC	no template control
MES	2-(N-morpholino) ethanesulfonic acid
MEN	MOPS-EDTA-NaAc-buffer
MOPS	morpholinopropan-sulfonic acid
mRNA	messenger RNA
MS medium	Murashige and Skoog medium
p	probability-value
PAA	polyacrylamide
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PEG	polyethylene glycol
PEP	plastid encoded plastid RNA polymerase
pH	*potentia hydrogenii*, -log [H+]
phy	phytochrome
pmol	picomol
PVP	polyvinylpyrrolidone
qRT-PCR	quantitative real-time PCR
r	red light
RNA	ribonucleic acid
RpoT	RNA polymerase of the T3/7-phage type
rRNA	ribosomal RNA
RT	reverse transcriptase
s	second(s)
SE	standard error
SEA	plant medium
SDS	sodium dodecyl sulfate
sol.	solution
TF	transcription factor
Tris	tris (hydroxymethyl)-aminomethane
tRNA	transfer RNA
UTP	uridine triphosphate
UV	ultra violet
v	version
V	Volt
v/v	volume per total volume
vol	volume
w/v	weight per volume (g/100ml)
ZBP	zeatin-binding protein

Acknowledgements

This work was financially supported by grants from the Deutsche Forschungsgemeinschaft (SFB429) and a Women Research Fellowship from the Institute of Biology (Humboldt-Universität zu Berlin). Several persons have contributed to these studies, and I wish to express my deepest gratitude to the following people:

Prof. Thomas Börner for giving me the opportunity to work in his group all these years, his strong support and for broadening my knowledge on various aspects of plant genetics.
Karsten Liere, Ph.D., for his valuable advice, his great sense of humor and for reviewing this thesis. Without him I would be totally ~~-l~~ost in translation".
Yan Zubo, Ph.D., for our interesting, shared studies. I wish to thank him for his willingness to discuss issues pertaining to our work at any time and any place.
Lars Dietzel (Friedrich-Schiller Universität Jena) and Stefanie Zintl (Freie Universität Berlin) for their kind cooperation during several experiments.
All current and former colleagues at the Department of Biology (Genetics) for their encouragement and good friendship. Especially, Conny Stock for her excellent technical advice and for making our lab such a pleasant place to work in. Johanna Sobanski for sharing her knowledge of molecular biology with me, Emilia Cincu for the flow cytometric analysis and Janina Apitz for helping me with the ~~-r~~eceptors". I wish to extend my warmest thanks to Arthur Guljamow.
Dear family for their interest in my academic work and for lots of support. Special thanks to Yvonne for being my friend for so many years and to my fiancé Oliver for sharing his life with me.

Curriculum Vitae

Publications and Conference Abstracts

PUBLICATIONS

Borsellino, L., Liere, K. and Börner, T. (2011) *Influence of light on organellar phage-type RNA polymerases transcript levels in* Arabidopsis thaliana*.* Manuscript in preparation.

Borsellino, L., Zubo, Y. and Börner, T. (2011) *Role of histidine kinase receptors in the regulation of plastidial transcription by cytokinin.* Manuscript in preparation.

CONFERENCE PRESENTATIONS

L. Borsellino (2011) *Influence of light on the transcription of genes for organellar RNA polymerases*. 24th Conference on Plant Molecularbiology, Dabringhausen, Germany.

L. Borsellino (2010) *Influence of light on the transcription of genes for organellar RNA polymerases*. Havel-Spree-Colloquium, Berlin, Germany.

Y. Zubo and L. Borsellino (2010) *Histidine kinase receptors are involved in regulation of chloroplast transcription by cytokinin.* 4th International Symposium of the SFB429, Potsdam, Germany.

L. Borsellino, K. Liere and T. Börner (2010) *Influence of light on the transcription of genes for organellar RNA polymerases.* XIth International Colloquium on Endocytobiology and Symbiosis, Tromso, Norway.

L. Borsellino (2009) *Influence of light on the transcription of RNA polymerases in* Arabidopsis*.* Havel-Spree-Colloquium 2009, Berlin, Germany.

CONFERENCE POSTERS

L. Borsellino, Y. Zubo and T. Börner (2011) *Regulation of chloroplast transcription by cytokinin.* Botanikertagung 2011, Berlin, Germany.

L. Borsellino, K. Liere and T. Börner (2010) *Influence of light on the transcription of genes for organellar RNA polymerases.* 4th International Symposium of the SFB429, Potsdam, Germany.

L. Borsellino, T. Preuten, K. Liere and Thomas Börner (2009) *Influence of blue-light on the transcript levels of RNA polymerases in* Arabidopsis thaliana *photoreceptor mutants*. XIII. Jahrestagung der Deutschen Sektion der Internationalen Gesellschaft für Endocytobiologie, Berlin, Germany.

L. Borsellino, T. Preuten, K. Liere and T. Börner (2009) *Influence of blue-light on the transcript levels of RNA polymerases in* Arabidopsis thaliana *photoreceptor mutants.* Joint Symposium of German Academy of Science Leopoldina, SFB 429 and SFB-TR1, Berlin, Germany.

K. Liere, L. Borsellino, T. Preuten, M. Swiatecka-Hagenbruch and T. Börner (2009) *The role of light in the expression of organellar phage-type RNA polymerases in* Arabidopsis. Botanikertagung 2009, Leipzig, Germany.

Eidesstattliche Erklärung

Hiermit versichere ich, die vorliegende Dissertation eigenständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet zu haben. Die dem Verfahren zugrunde liegende Promotionsordnung ist mir bekannt.

Die Dissertation wurde in der jetzigen oder einer ähnlichen Form bei keiner anderen Hochschule eingereicht und hat noch keinen sonstigen Prüfungszwecken gedient.

Berlin, 06. September 2011

…….

(Liliana Borsellino)