Identification of Proteins Associated with Spatial-Specific Phytochrome-Mediated Light Signaling in *Arabidopsis thaliana* by Liquid Chromatography-Tandem Mass Spectrometry

Sookyung Oh¹ and Beronda L. Montgomery¹,²

¹Department of Energy–Plant Research Laboratory, Michigan State University, East Lansing, MI, 48824-1312;
²Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824-1319

**ABSTRACT**

Photosensory phytochromes perceive mainly red and far-red light and utilize a linear tetrapyrrole chromophore, phytochromobilin, for their photoactivity in plants. Although phytochromes have been extensively studied for light-dependent regulation of numerous developmental processes, our understanding of the molecular mechanisms responsible for distinct organ- and tissue-specific phytochrome responses is still limited. Recent studies using transgenic *Arabidopsis thaliana* plants expressing a gene that encodes the biliverdin IXα reductase (BVR) enzyme, which reduces the biosynthesis and accumulation of phytochromobilin, and thus inactivates phytochromes, have led to advances in probing tissue-specific roles of phytochromes in plant development. We performed one-dimensional SDS-PAGE, followed by protein identification and peptide quantification using liquid chromatography-tandem mass spectrometry (LC/MS-MS) to identify proteins that accumulate differentially in transgenic *Arabidopsis* lines with mesophyll-specific phytochrome deficiencies (i.e., CAB3::pBVR2 plants) compared to wild-type (WT). We identified the large subunit of Rubisco (RbcL) and small subunit of Rubisco (RbcS), which accumulated to lower levels in CAB3::pBVR2 relative to WT under continuous far-red light. We found that Beta-glucosidase proteins (BGLUs) accumulated highly in the CAB3::pBVR2 line under these conditions. RT-PCR and microarray analyses showed a positive correlation between the expression of the target genes and the accumulation of their products in BVR lines. We conclude that RbcL, RbcS, and BGLU18 are targets of mesophyll-specific phytochrome-mediated light signaling under far-red conditions.

**INTRODUCTION**

As sessile organisms, plants have evolved several photoreceptors to monitor and respond to diverse light environments. Plants monitor several different properties of available light, including intensity, wavelength, duration, and direction. Phytochromes are an extensively-studied photoreceptor family, which mainly perceive red (R) and far-red (FR) light (Schepens et al., 2004; Franklin and Quail, 2010; Kami et al., 2010). It has been demonstrated that the subcellular localization of phytochromes is important for the associated light-dependent responses (Nagy et al., 2001; Nagatani, 2004). Phytochromes move from the cytosol into the nucleus in a light-dependent manner and in the nucleus they can interact with transcription factors and regulate gene expression.

**Phytochrome Functions in Plants**

Phytochromes are involved in light-dependent regulation of numerous developmental processes such as seed germination, hypocotyl elongation responses, hypocotyl gravitropism, shade avoidance, and flowering (Franklin and Quail, 2010; Kami et al., 2010). It has been demonstrated that the subcellular localization of phytochromes is important for the associated light-dependent responses (Nagy et al., 2001; Nagatani, 2004). Phytochromes move from the cytosol into the nucleus in a light-dependent manner and in the nucleus they can interact with transcription factors to regulate gene expression.
factors, including phytochrome-interacting factors (PIFs), resulting in the regulation of light-responsive genes (reviewed by Castillon et al., 2007). Phytochromes function through PIFs to regulate a number of light-dependent responses, including seed germination, chlorophyll accumulation, hypocotyl and cotyledon development and gravitropic responses (Shin et al., 2009). Specifically, phytochrome-dependent regulation of PIFs is involved in the control of hormone-dependent seed germination (Oh et al., 2009). Also, it has been demonstrated that phytochromes regulate hypocotyl gravitropism through PIF-dependent effects on endodermal amyloplast development (Kim et al., 2011). Phytochromes also appear to have roles in the cytosol, which include blue-light dependent negative gravitropism and red-light enhanced phototropism (Rössler et al., 2007). The diverse roles of phytochromes in various physiological responses in plants recently have been reviewed extensively (Franklin and Quail, 2010; Kami et al., 2010).

**Phytochrome Accumulation in Specific Plant Tissues**

In addition to light-dependent differences in subcellular phytochrome localization, the localization of phytochromes at the tissue and organ level also has been studied (for review see Nagatani, 1997). Early reports established the differential accumulation of phytochromes in distinct plant tissues, e.g., phytochromes accumulate differentially in the epicotyl hook, cotyledons, and root tips in pea seedlings (Furuya and Hillman, 1964). Such observations have been substantiated further by phytochrome promoter fusion studies, which indicate that distinct phytochrome isoforms display discrete tissue- and organ-specific patterns of expression in a range of plant species (Somers and Quail 1995a; 1995b; Adam et al., 1996; Goosey et al., 1997; Tóth et al., 2001), as well as by immunolocalization of phytochrome holoproteins (Sharrock and Clack, 2002). These observations began to implicate distinctive roles of phytochromes in specific tissues and organs.

**Phytochromes Regulate Discrete Responses in Different Tissues and Organs**

The spatiotemporal biochemical and physiological roles of phytochromes in light-dependent plant responses also have been explored extensively (for review see Bou-Torrent et al., 2008; Montgomery, 2008). At the physiological level, a number of early studies utilized detached or foil-covered plant parts or site-specific microbeam irradiation to photoactivate phytochromes in discrete plant tissues and to assay for distinct phytochrome-dependent responses in distal sites (Piringer and Heinze, 1954; Klein et al., 1956; De Greef et al., 1971; De Greef and Caubergs, 1972a; 1972b; De Greef and Verbelen, 1972; Teperer and Bonnett, 1972; Black and Shuttleworth, 1974; Oelze-Karow and Mohr, 1974; Caubergs and De Greef, 1975; De Greef et al., 1975; Lechnary, 1979; Powell and Morgan, 1980; Mandoli and Briggs, 1982; Nick et al., 1993). Such studies, e.g., experiments using foil to cover specific portions of cucumber seedlings during irradiation (Black and Shuttleworth, 1974), led to the observation that activation of phytochromes in cotyledons could inhibit the elongation of hypocotyls through interorgan signaling in response to light. Other classical examples of cell- and tissue-specific, as well as interorgan, phytochrome-mediated responses emerged, including the perception of light by leaf-localized phytochromes that results in the regulation of the photoperiodic induction of flowering that produces a transition from vegetative to reproductive growth at the meristem (King and Zeevaart, 1973). Although such early physiological approaches have broadened our knowledge of spatial-specific phytochrome responses, our understanding of the molecular mechanisms responsible for such tissue-specific phytochrome responses is still limited. These limitations persist partially due to a lack of molecular tools for probing the role(s) of phytochromes in a tissue-specific manner.

**Light Perception by Photoreceptors Regulates Gene Expression in a Tissue-Specific Manner**

Through recent studies, insight into light-dependent regulation of gene expression at the organ- and tissue-specific level has increased. Tissue-specific expression of photoreceptors identified distinct tissues in which cryptochromes (Endo et al., 2007) and phytochromes (Endo et al., 2005) are required for the regulation of specific responses. Also, distinct subsets of genes have been shown to be controlled by light in different tissues, e.g., cotyledon, hypocotyls and roots in both rice and Arabidopsis (Jiao et al., 2005; 2007). Thus, it appears that specific factors are regulated in distinct tissues and organs downstream of light perception by photoreceptors. Furthermore, reverse genetic studies of genes regulated in response to light resulted in the identification and characterization of factors implicated in hypocotyl-localized phytochrome responses (Khanna et al., 2006).
Phytochrome Depletion in Transgenic Plants

Recently, great advances have been made in the elucidation of the molecular mechanisms responsible for tissue- and organ-specific phytochrome responses using transgenic plants expressing a gene that encodes the mammalian enzyme biliverdin IXα reductase (BVR). Constitutive expression of BVR in transgenic Arabidopsis and tobacco plants using a strong plant promoter, i.e., the promoter of the 35S RNA from Cauliflower mosaic virus (i.e., CaMV 35S; Odell et al., 1985), resulted in the inactivation of the precursors of the phytochrome chromophore, and alteration of light-dependent responses associated with reduced phytochrome activity (Lagarias et al., 1997; Montgomery et al., 1999; Montgomery et al., 2001). These responses mirrored the responses that have been observed in natural phytochrome chromophore-deficient mutants. Experiments with transgenic plants selectively expressing the BVR gene using tissue-specific promoters, and consequently in which the function of phytochromes have been inactivated in a tissue-specific manner, have allowed successful probing of the spatial-specific roles of phytochromes in plant development (Montgomery, 2009; Warnasooriya and Montgomery, 2009; Warnasooriya et al., 2011; Costigan et al., submitted). BVR expression in plastids of mesophyll tissues of Arabidopsis was achieved using the CAB3 promoter (i.e., CAB3::pBVR), and resulted in the disruption of FR-dependent responses, including the inhibition of hypocotyl elongation and the stimulation of anthocyanin accumulation, suggesting roles for mesophyll-localized phytochrome A (phyA) in the regulation of FR-dependent responses (Warnasooriya and Montgomery, 2009). CAB3::pBVR lines also have disruptions in red- and blue-light-dependent inhibition of hypocotyl elongation and anthocyanin accumulation responses (Montgomery, 2009; Warnasooriya and Montgomery, 2009; Warnasooriya et al., 2011). Inactivation of phytochromes in the shoot apical meristem in transgenic Arabidopsis plants, i.e. plants expressing BVR under the control of the MERI5 promoter, results in an enlarged leaf phenotype and an increased leaf number specifically under short-day photoperiods, suggesting a photoperiod-dependent role of meristem-specific phytochromes in the regulation of leaf initiation and growth (Warnasooriya and Montgomery, 2009).

Identifying the Molecular Effectors that Mediate Tissue- and Organ-Specific Phytochrome Responses

Using microarray analysis to identify genes which were differentially expressed in FR-light-grown 35S::pBVR seedlings relative to CAB3::pBVR seedlings, we identified several genes involved in mesophyll-specific phytochrome responses (Warnasooriya, Oh, and Montgomery, unpublished data). These studies are providing insight into the molecular mechanisms underlying spatial-specific phytochrome responses during Arabidopsis seedling development. In this study, we report parallel experiments to identify specific proteins that accumulate differentially in No-0 wild-type (WT) relative to 35S::pBVR, CAB3::pBVR, or MERI5::pBVR transgenic lines using SDS-PAGE analysis followed by enzymatic digestion, liquid chromatography-tandem mass spectrometry (LC-MS/MS) and database searching. We found that the large subunit of Rubisco (RbcL) and small subunit of Rubisco (RbcS) accumulated to lower levels in CAB3::pBVR lines than in No-0 WT or other BVR lines, whereas Beta-glucosidase proteins (BGLUs) accumulated highly in CAB3::pBVR lines relative to No-0 WT or other BVR lines. Using microarray data and RT-PCR analysis, we confirmed a positive correlation of protein accumulation with the accumulation of mRNA of the genes encoding the identified proteins in CAB3::pBVR lines. These data suggest that RbcL, RbcS, and BGLUs are targets of mesophyll-specific, phytochrome-mediated light signaling and potentially involved in tissue-specific phytochrome responses during seedling development.

Identification of Proteins Differentially Accumulated in BVR Lines

To identify proteins responsible for tissue- and organ-specific-phytochrome responses, we performed profiling of proteins differentially accumulated in BVR lines. Total soluble proteins were extracted from No-0 WT (hereafter WT), 35S::pBVR3 (35S), CAB3::pBVR2 (CAB3), and MERI5::pBVR1 (MERI5) seedlings grown on 1× MS medium containing 1% sucrose at 22 °C in FR light at 5 μmol m⁻² sec⁻¹ for 14 days. Soluble proteins (25 μg) were resolved on 8 % or 12 % (w/v) polyacrylamide SDS-PAGE gels. At least three distinctive protein bands were detected visually: Proteins from band 1 or 2 accumulated to lower levels in CAB3 lines, whereas proteins from band 3 accumulated highly in CAB3 lines (Figure 1). These altered patterns of protein accumulation that were observed on gels were reproducible in at least three biological replicates. The
three protein bands labeled 1 through 3 in the gels from WT and CAB3 samples (Figure 1) were excised and subjected to tryptic digestion followed by LC-MS/MS and database searching for homology to known proteins. All procedures for LC-MS/MS were performed at the Research Technology Support Facility (RTSF) at Michigan State University. The Mascot search engine (Matrix Science, Inc., Boston, MA) was used to match MS/MS spectra to peptide sequences. Proteins identified with more than 99.7 % of protein identification probability by using Scaffold software (Proteome Software, Inc., Portland, OR) are listed in Table 1. Spectral counts (i.e., number of spectra) have been used as a good measure of relative abundance among different samples (Liu et al., 2004). We assessed the number of spectra, % of spectra, and % of coverage for the comparison of candidate proteins in WT relative to CAB3 (Table 1).

Consistent with data from SDS-PAGE gel analyses, the large subunit of Rubisco (RbcL), which is labeled protein band 1, was less abundant in CAB3 relative to WT when comparing spectral counts, i.e., 106 vs. 176, respectively (Table 1). The LC-MS/MS data for RbcL was reproducible when tested using two biologically independent samples. As predicted based on the protein gel, the molecular mass of RbcL was 53 kDa (Figure 1). Proteins from band 2 were identified as the small subunit of Rubisco (RbcS; Table 1). Two RbcS proteins encoded by At1g67090 and At5g38410 accumulated to lower levels in CAB3 relative to WT – i.e. spectral counts for WT vs. CAB3 of 83 vs. 48 for At1g67090 and 13 vs. 8 for At5g38410. Data from mass spectrometry analysis for RbcS was reproducible in two biologically independent replicates. Whereas the predicted molecular mass of RbcS was 20 kDa (Table 1), the apparent molecular mass of RbcS on the protein gel was approximately 10 kDa (Figure 1). This observation indicates post-translational modification of Rubisco proteins, e.g. proteolytic processing of the N-terminal portion as previously described (Houtz et al., 2008).

Proteins from band 3, which were highly accumulated in CAB3 relative to WT as observed on SDS-PAGE gels (Figure 1), were identified as BGLUs in our LC-MS/MS analysis (Table 1). The Arabidopsis genome encodes at least 48 Beta-glucosidase proteins (BGLUs) and BGLU23, BGLU22, BGLU21, and BGLU 18 are most closely related (Xu et al., 2004; Ogasawara et al., 2009). All of the closely related BGLU23, BGLU22, BGLU21, and BGLU18 proteins were found to be more abundant based on spectral count in CAB3 than in WT—112 vs. 68 for BGLU23, 6 vs. 0 for BGLU22, 3 vs. 0 for BGLU21, and 5 vs. 2 for BGLU18 (Table 1).
Table 1. Identification of proteins differentially accumulated in wild-type (WT) and CAB3::pBVR2 (CAB3) lines using liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. Proteins listed were identified with probabilities of ≥ 99.7% after spectral searches with the Mascot database search engine (Matrix Science, Inc.) to identify peptides and analysis with Scaffold software (Proteome Software, Inc.).

<table>
<thead>
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<th>Band No.</th>
<th>AGI No.</th>
<th>Annotation (Mass)</th>
<th>Samples</th>
<th>No. of spectra</th>
<th>% of spectra</th>
<th>% of coverage</th>
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<td>WT</td>
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</tr>
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<td></td>
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<td></td>
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<td>CAB3</td>
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Expression Patterns of Genes Encoding Differentially Accumulated Proteins in BVR Lines Are Also Altered

We previously performed comparative gene expression profiling studies using BVR lines grown on MS medium for 7 days in FR light (Warnasooriya, Oh, and Montgomery, unpublished data). Using our microarray data, which was produced in triplicate and subjected to per chip normalization, the expression of genes encoding the candidate proteins identified from our LC-MS/MS analysis has been explored (Figure 2). AtCg00490 encoding RbcL was downregulated in CAB3 lines compared to 35S and WT – i.e., 3.5- and 6.4-fold downregulated, respectively (Figure 2A). The two genes encoding RbcS proteins, i.e., At1g67090 and At5g38410, were moderately downregulated by 1.6 fold in CAB3 compared to WT or 35S lines, which were not significantly different from each other (Figure 2B). Genes encoding the four identified BGLUs tended to be upregulated in BVR lines compared to WT (Figure 2C). Most strikingly, BGLU18 was highly upregulated in CAB3 lines relative to 35S and WT, i.e., 4.6- and 7.5-fold, respectively.

To verify the expression of the genes queried in our microarray data set, we performed RT-PCR analyses (Figure 3). Also, in some cases microarray data did not provide the expression of specific genes. For example, the two RbcS-encoding genes and the genes encoding BGLU21 and BGLU22 could not be distinguished. Thus, it was very important to verify the expression of those genes using RT-PCR analysis. Primer pairs for candidate genes and a control gene encoding an ubiquitin-conjugating enzyme 21 (UBC21) were designed using AtRTPrimer (Han and Kim, 2006). RT-PCR analyses confirmed the expression patterns of our candidate genes: AtCg00490 and At1g67090 encoding RbcL and RbcS, respectively, were downregulated in CAB3 lines compared to WT or 35S lines; whereas At1g52400 encoding BGLU18 was highly expressed in CAB3 relative to WT (Figure 3). Altogether, the results from microarray and RT-PCR analyses indicated a positive correlation between gene expression and associated protein accumulation in BVR lines.

Expression of Candidate Genes in Various Tissues and Organs

To obtain more insight into the expression of the candidate genes in different tissues and at distinct developmental stages, we constructed heat maps using AtGenExpress public Arabidopsis microarray data set. RBCL was not represented in this data set. Notably, the expression pattern of RBCL and BGLU18...
in various tissues was quite similar (Figure 4). RBCS and BGLU18 are expressed abundantly in aerial tissues, including hypocotyls, cotyledons, and leaves. Unlike BGLU18, BGLU23 and BGLU22/21 are upregulated highly in roots (Figure 4). BGLU23 is also abundant in hypocotyls and the shoot apex (Figure 4). These observations correspond well to BGLU18 being affected more significantly at the level of expression than the other BGLU-encoding genes in CAB3:pBVR lines where phytochromes are inactivated in cotyledons and leaves—i.e. the site of BGLU18 upregulation.

**Potential Roles of RBCL, RBCS, and BGLU18 in Mesophyll-Specific Phytochrome Responses**

In the stroma of chloroplasts, Rubisco catalyzes the oxygenation of ribulose-1,5-bisphosphate in the photosynthetic pathway and carboxylation of the same substrate during the Calvin cycle (reviewed by Jensen and Bahr, 1977). Rubisco is one of the most abundant proteins comprised of RbcL and RbcS to form a massive hexadecameric protein structure (Baker et al., 1975). In Arabidopsis, RbcL protein is encoded by a single chloroplast-localized gene, A1Cg00490 (Bedbrook et al., 1979), whereas RbcS proteins are encoded by a nuclear multigene family, including At1g67090 and At5g38410 (Dean et al., 1989).

We found that the level of accumulation of RbcL and RbcS proteins in CAB3 was lower than in WT, 35S, or MERI5 lines (Figure 1). In CAB3 lines, the genes encoding those two proteins were also downregulated, suggesting a role of mesophyll-specific phytochromes in the positive regulation of RBCL and RBCS genes (Figures 2, 3). It has been demonstrated that phytochromes are involved in the induction of the expression of RBCL and RBCS at the transcriptional level in several species (e.g. Sasaki et

![Figure 2](image1.png)

**Figure 2.** Selection of candidate genes differentially expressed in BVR lines using microarray data. Expression levels (Signal values) of genes encoding RBCL, RbcS, and BGLU proteins identified from liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis are shown. The data shown are from Arabidopsis ATH1 (Affymetrix) microarray experiments with three independent replicates (Warnasooriya, Oh, and Montgomery, unpublished data). A. RBCL: Large subunit of ribulose 1,5-bisphosphate carboxylase (Rubisco); B. RBCS: Small subunit of Rubisco; C. BGLU: Beta-glucosidase. Note that for RBCS or BGLU22/21, a single probe set from microarray data represents multiple genes.

![Figure 3](image2.png)

**Figure 3.** RT-PCR analysis of candidate genes encoding RBCL, RBCS, and BGLU18 proteins identified from liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. 7 day-old seedlings of wild-type (WT), 35S::pBVR3 (35S), and CAB3::pBVR2 lines (CAB3) grown under constant far-red light were used. RT-, no reverse transcriptase (RT) negative control. UBC21 gene encoding a ubiquitin-conjugating enzyme was used as an internal control to demonstrate relative quantity and quality of the cDNA template for RT-PCR. Results shown are representative of two independent biological replicates.
Oh and Montgomery -- Arabidopsis Phytochrome Protein

Furthermore, Antipova et al. (2004) demonstrated that FR light induces the accumulation of RbcL protein and antisense suppression of PHYA in transgenic tobacco plants results in a reduced level of accumulation of RbcL protein, indicating a role for phyA in the FR light-mediated photoregulation of the synthesis of RbcL proteins. Here, we further define the mesophyll-specific pool of phyA as critical for regulation of RbcL accumulation in Arabidopsis. It also has been shown that At1g67090, which encodes a RbcS protein, is highly expressed in mesophyll tissues and upregulated in cotyledons and hypocotyls in response to blue, red, and far-red light in Arabidopsis (Sawchuk et al., 2008). Consistent with reduced levels of RbcS protein in CAB3 lines (Figure 1), expression of At1g67090 was downregulated in those lines (Figures 2 and 3). Striking phenotypes observed in CAB3 lines include defects in FR-dependent inhibition of hypocotyl elongation and anthocyanin accumulation, indicating roles of mesophyll-localized phyA in FR responses (Warnasooriya and Montgomery, 2009). Here, we highlight another mesophyll-localized, FR-dependent phenotype regulated by phyA—the regulation of expression of RBCS and RBCL genes and accumulation of their proteins.

We found that BGLU proteins were highly accumulated in CAB3 lines and the expression of BGLU18 gene was correlated with the observed increased protein accumulation (Figures 1, 2 and 3), suggesting a role of mesophyll-specific phytochrome in the negative regulation of the BGLU18 gene. BGLUs catalyze the hydrolysis of cellobiose, a unit of cellulose, or other disaccharides with release of glucose and play important roles in many biological processes, including chemical defense against pathogens (Morant et al., 2008), lignification (Dharmawardhana and Ellis, 1998), degradation of cell wall materials (Leah et al., 1995), and hydrolytic hormone release (Lee et al., 2006). Interestingly, it has been demonstrated that an oat BGLU protein, also named Avenacosidase, co-purifies with phytochrome (Gus-Mayer et al., 1994; Parker et al., 1995). However, there has been no further evidence reported of a functional role of BGLUs in phytochrome-mediated light signaling.

The Arabidopsis genome encodes 48 BGLUs and eight of them, i.e., BGLU18 – BGLU25, are placed in the same phylogenetic clade (Xu et al., 2004). Identification of BGLU23, BGLU22, BGLU21, and BGLU18 in our mass spectrometry analysis suggests a functional redundancy of those proteins in spatial-specific phytochrome-mediated light signaling (Table 1). Among them, only the expression BGLU18 gene was heavily upregulated (~5 fold) in CAB3 compared to 35S lines (Figures 2 and 3), indicating a regulation of BGLU18 by mesophyll-specific phytochromes. Interestingly, the expression pattern of BGLU18 in different Arabidopsis tissues was roughly correlated with that of RBCS, i.e., RBCS and BGLU18 were upregulated in mesophyll-abundant organs such as cotyledons and leaves, whereas BGLU23, BGLU22, and BGLU21 were abundantly expressed in roots (Figure 4), suggesting a distinct mesophyll-specific

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**Figure 4.** Heat map showing the expression of RBCS and BGLUs in different Arabidopsis tissues and organs at various developmental stages. Mean-normalized values from AtGenExpress microarray data set (http://www.weigelworld.org) and BAR Heatmapper Plus (http://esc4037-shemp.csb.utoronto.ca/welcome.htm) were used for heat map. Log-transformed microarray expression data is displayed; Red represents high expression and yellow denotes low expression. The name of tissues/organs (tissue) and age of samples (day) are indicated. Note that microarray data for RBCL was not available in AtGenExpress.

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<table>
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<tr>
<th>Tissue</th>
<th>Expression of RBCS</th>
<th>BGLU23</th>
<th>BGLU22/21</th>
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<td>7d</td>
<td>17 to 21d</td>
<td>28 to 56d</td>
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<td>7d</td>
<td>17 to 21d</td>
<td>28 to 56d</td>
<td>0</td>
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<tr>
<td>Seed</td>
<td>7d</td>
<td>17 to 21d</td>
<td>28 to 56d</td>
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</table>
regulation of BGLU18 by phytochrome-mediated light signaling.

It has been shown that BGLU18 is induced by herbivory, methyl jasmonate, and wounding and its protein accumulates in endoplasmic reticulum bodies formed directly at the wounding site on cotyledons, indicating a role of BGLU18 in plant defense responses (Stotz et al., 2000; Ogasawara et al., 2009). Since a positive correlation between the specific activity of BGLU and the rate of cell wall elongation has been observed in pea seedlings (Murray and Bandurski, 1975), we propose that defense-related BGLU18 could also be involved independently in the regulation of cell elongation (e.g., inhibition of hypocotyl elongation) during seedling development. This involvement could be through the impact of BGLU18 on the cell wall, or perhaps through light-dependent regulation of the hydrolytic release of a hormone impacting cellular elongation. Interestingly, inactivation of phytochrome in CAB3 lines resulted in a defect in the inhibition of hypocotyl elongation (Warnasooriya and Montgomery, 2009) and in the present studies, BGLU18 proteins accumulated highly in CAB3 lines, compared with other BVR lines (Figure 1). Thus, it appears that an increased accumulation of the BGLU18 enzyme is associated with an elongation of hypocotyls – thus in normal seedling development expression of BGLU18 may be regulated negatively by mesophyll-specific phytochromes during the light-dependent inhibition of hypocotyl elongation.

SUMMARY AND CONCLUSION

We applied SDS-PAGE gel analysis coupled with mass spectrometry to identify key protein components regulated by mesophyll-specific phytochromes. Our mass spectrometry-based protein analysis identified three proteins differentially accumulated in BVR lines. In CAB3, transcript abundance of RBCS and RBCL and associated protein abundance were low relative to WT and other BVR lines with distinct tissue-specific disruptions in phytochrome accumulation. However, BGLUs accumulated to higher levels and transcript accumulation of BGLU18 was upregulated in CAB3. Since mesophyll-specific phytochromes are inactivated in CAB3 lines, we hypothesize that the genes encoding RbcS, RbcL, and BGLU18 proteins are targets of mesophyll-specific phytochrome signaling pathways. Further investigations using genetic approaches to characterize mutants for genes encoding these proteins and/or two-dimensional gel electrophoresis coupled with mass spectrometry analysis to identify additional candidate proteins differentially accumulated in various BVR lines will allow a better understanding of the molecular mechanisms underlying spatial-specific phytochrome-mediated light signaling and the associated regulation of distinct phytochrome-dependent responses.

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