



Society for Experimental Biology Annual Main Meeting 28th June – 1st July 2009, Glasgow, UK

P4 – PHOTOMORPHOGENESIS

P4.1

09:00 Tuesday 30th June 2009

Seedling photomorphogenesis as seen through the eyes of a computer

Edgar P. Spalding (University of Wisconsin)

The field of photomorphogenesis was greatly advanced by the discovery of *Arabidopsis* mutants with very telling phenotypes such as long or short hypocotyls. Much was learned by studying the dramatic phenotypes even with coarse methods because the difference was so large. But with the development of high-resolution tools for measuring hypocotyl growth and shape, more detailed information about the phenotypes including temporal information important to mechanistic models of photoreceptor function could be extracted. For example, analysis of electronic images of *Arabidopsis* seedlings undergoing de-etiolation showed that the phot1 blue light receptor initiates a 30-min phase of growth inhibition that precedes the sustained cry1-mediated phase. The next generation of image analysis algorithms, largely in the form of the HYPOTrace program, further facilitated high-resolution growth and shape measurements. These newer methods were used to quantify the effect of manipulating nuclear localization of the cry1 photoreceptor on blue light-induced growth inhibition. It was found that nuclear cry1, without detectable contribution from cytoplasmic cry1, was responsible for blue light-induced suppression of hypocotyl growth. These studies on cry1 responses have begun to connect with our studies of polar auxin transport mediated by ABC transporters in the multidrug resistance-like family. Mutations in the ABCB19 transporter, which reduce basipetal auxin transport by 80%, partially suppress the fast growth of the cry1 hypocotyl, apparently by reducing hypocotyl auxin signaling. Mutations in cry1 increase ABCB19 protein levels and hypocotyl auxin, contributing to their faster growth. Uncovering these interactions required mutants with phenotypes and machine-vision tools for quantifying them.

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P4.2

09:50 Tuesday 30th June 2009

Impending effects of transpiration in blue light regulation of leaf growth

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Light quality, drives several photomorphogenetic responses in plants such as leaf growth. A decrease in blue irradiance in the incident light leads to an increase in leaf elongation rate and final leaf length. The effects of blue light could be mediated by water flux into the growing zone since blue light has a direct effect on stomata aperture and consequently on plant transpiration. Indeed, several studies have demonstrated the effects of changes in transpiration rate on leaf elongation rate but little is known about the control of leaf elongation by water flux under low blue light conditions.

The objective of this study was to determine the contribution of plant transpiration for blue light control of leaf elongation. Plants of tall fescue were submitted to neutral or to blue lacking light treatment. Leaf elongation was measured before and after changes in light conditions by using LVDT-sensors. Plant transpiration was monitored continuously by the gravimetric method (balance).

In response to lack of blue light, leaf elongation rate was increased by about 11%, conversely, transpiration rate and stomatal conductance were decreased by 25% and 33%, respectively. These inverse changes in leaf elongation and plant transpiration exhibit a specific time-response pattern, were not observed in control plants. These results suggest that the effects of blue light on leaf growth in grasses are mediated by the effects of blue light on transpiration.

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P4.3**10:40 Tuesday 30th June 2009****Blue light suppression of CIB1 protein degradation in *Arabidopsis***

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Cryptochromes (CRY) are photolyase-like blue-light receptors that mediate light responses in plants and animals. How plant cryptochromes act in response to blue light is not well understood. We have recently reported (Science 322: 1535–1539) a signaling protein called CIB1 (cryptochrome-interacting bHLH 1). CIB1 is a bHLH transcription factor that interacts with CRY2 (cryptochrome 2) in a blue light-specific manner. CIB1 acts with additional CIB1-related proteins to promote CRY2-dependent activation of FT gene expression and floral initiation and. CIB1 binds to the promoter region of the FT gene in vivo. We proposed that the blue light-dependent interaction of cryptochrome(s) with CIB1 and CIB1-related proteins represents an early photoreceptor signaling mechanism in plants. Consistent with the hypothesis that CIB1 is involved in signaling of blue light receptor CRY2, we discovered that CIB1 protein expression is regulated specifically by blue light. CIB1 protein is degraded in the absence of blue light, via an ubiquitin/proteasome pathway. However, CIB1 proteolysis is suppressed in response to blue light, resulting in accumulation of CIB1 protein in plants exposed to blue light. We hypothesize that blue light suppression of CIB1 degradation may enhance the blue light signal perceived by cryptochromes.

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P4.4**11:20 Tuesday 30th June 2009****Superoxide production in the *Arabidopsis* root tip depends on light**

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Light and reactive oxygen species (ROS) are crucial factors that regulate plant growth and development. The light-regulated processes in hypocotyls have been studied extensively, however, for roots, our understanding of the photomorphogenesis processes is limited. It is known that superoxide is necessary for the root hairs tip growth and for the root elongation. The widely used indicator for the superoxide detection is the nitroblue tetrazolium (NBT). In 5 days-old *Arabidopsis* seedlings, grown in sterile conditions under 16/8 h photoperiod the typically root growth zones dependent NBT distribution could be detected. However we haven't found NBT staining of the root elongation zone in the dark-grown seedlings, but only slight NBT staining in the region with the high density of the root hairs. We suggest that different enzymes are responsible for the superoxide generation in the root hairs and in the cells of root elongation zone. The dark-grown seedlings exhibit extensive NBT staining of the root tip if kinetin was present in the growth medium. NBT staining was also observed in the root tip of dark-grown seedlings grown in the presence of sodium nitroprusside – an NO donor that is known as a stimulator molecule in plant photomorphogenesis. CuSO₄ that typically result in increasing in the levels of ROS also induce extensive root tip NBT staining in dark-grown seedlings. We suggest that the ability to generate superoxide in the

root elongation zone is the result of the photomorphogenesis changes in the *Arabidopsis*.

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P4.5**11:40 Tuesday 30th June 2009****Regulatory processes underscoring the light control of shoot meristem activity and leaf initiation**

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Dark-grown seedlings exhibit repressed shoot apical meristem activity, and arrested, incipient leaf primordia. Upon exposure to light, leaves rapidly expand and differentiate. An in-depth examination of this transition should allow the uncovering of phenomena at very early stages in leaf development. We dissected shoot apices of *Arabidopsis* seedlings grown in the dark, and over a time course following the transfer to light, and carried out microarray analysis using ATH1 arrays. We compared the shoot apical gene expression programme with that of cotyledons. Several layers of regulation were observed. The clearest early transcriptional responses were associated with plant hormones and with protein ubiquitination. Gene expression signatures revealed an early shoot apex-specific down-regulation of the response to auxins and ethylene, and an elevation of gibberellin and cytokinin action. The cytokinin action signature followed the drop in auxin responses and coincided in time with a highly synchronous initiation of cellular growth and cell cycle activity. The early hormonal responses were transient, with auxin responses rising dramatically at the time of leaf primordia expansion. Preliminary examination confirms the rapid, inverse regulation of auxin- and cytokinin-responsive promoters at the apex. Another layer of regulation, likely to be associated with the synchronous cell cycle re-entry, involved complementary changes in the protein levels of transcription factors of the E2F family. Such changes depended on the function of the ubiquitin ligase or ubiquitination regulators COP1, COP signalosome and DET1. Light signalling provides a highly focused problem in which to unravel the fundamental processes of leaf initiation and growth.

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P4.6**13:40 Tuesday 30th June 2009****Phytochromes coordinate hormone signalling to promote seed germination**

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Phytochromes are red and far-red light receptors that regulate various physiological and developmental processes ranging from seed germination, photosynthetic capacity, to flowering in plants. Among these processes, seed germination provides a good model system to investigate how phytochromes regulate these processes in concert with

various plant hormones. We previously showed that phytochromes promote seed germination by destabilizing PIL5, a phytochrome-interacting bHLH transcription factor, that inhibits seed germination by regulating the expression of GA- and ABA-biosynthetic and signaling genes. To further investigate the role of PIL5 on seed germination, we performed ChIP-Chip and microarray analyses. By comparing the ChIP-chip and microarray data, we found that PIL5 regulates 166 genes by directly binding to their promoters. Many of these PIL5-regulated direct target genes encode transcription regulators involved in not just GA and ABA, but many other hormone signaling, while some encode enzymes involved in cell wall modification. Consistent with the regulation of various hormone signaling genes, the *pil5* mutant display altered germination frequencies in response to various hormones. Taken together, our data indicate that PIL5 inhibits seed germination not just through GA and ABA, but by coordinating hormone signals and modulating cell wall properties in imbibed seeds.

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P4.7

14:30 Tuesday 30th June 2009

Regulatory networks for the shade avoidance response

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A plant growing in the field has the unique ability to sense the presence of other plants growing nearby and adjust its growth rate accordingly. The early perception of neighbour proximity depends on the detection of light quality changes. Within a vegetation community, the ratio of red (R) to far-red (FR) light is lowered by the absorption of R light by photosynthetic pigments. This light quality change is perceived as an unambiguous signal of the proximity of neighbours through the phytochrome system. Upon sensing a low R/FR ratio, a shade avoiding plant reacts very rapidly and enhances elongation growth at the expenses of leaf and root development. If the plant succeeds in the attempt to overgrow its neighbours, the shade avoidance response is rapidly reverted through phytochrome photoconversion.

Many lines of evidence connect the phytohormone auxin to the rapid elongation response provoked by light quality changes. Our recent work establishes that auxin plays a pivotal role in leaf and root responses to low R/FR as well, suggesting that this phytohormone may act as a coordinator of plant growth responses to environmental light quality changes.

Persistency of a low R/FR signal results in attenuation of the shade avoidance response, which is controlled in part through the action of the bHLH HFR1/SICS1 transcription factor gene. Our recent data demonstrate that HFR1/SICS1 functions in the phyB signal transduction pathway and acts in concert with other transcription factors modulated through phyA in the adaptation of the plant to this unfavourable environmental condition.

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P4.8

15:30 Tuesday 30th June 2009

Structural basis of phyA-specific properties

Akira Nagatani (Kyoto University)

Phytochromes are encoded by a small multigene family. Among the members, phytochrome A (*phyA*) is the most specialized. Unlike other phytochromes, *phyA* is degraded rapidly in the light. *PhyA* mediates atypical phytochrome responses such as very low fluence responses (VLFR) and far-red (FR) high-irradiation response (FR-HIR). In both the cases, *phyA* can trigger the responses under conditions in which the active Pfr *phyA* exist at a very low level. Hence, *phyA* has evolved as a highly-sensitized photoreceptor.

Toward understanding of the structural basis for the above properties, we constructed chimeric phytochromes between *phyA* and phytochrome B (*phyB*). The phytochrome molecule was divided into four parts, namely N-terminal extension plus PAS (N-PAS), GAF, PHY and the C-terminal half, according to the domain structure. These domains were shuffled between *phyA* and *phyB* (in total, 16 constructs) and expressed in the *Arabidopsis phyAphyB* mutant under the control of the viral 35S promoter.

The physiological analyses of the above lines revealed *phyA* sequences required for each of *phyA*-specific properties. The main conclusions are as follows. 1) The nuclear localization activity under FR was solely determined by the source of the N-PAS domain. 2) For the hypocotyl FR-HIR, both the N-PAS and PHY domain had to be from *phyA* sequence. 3) The instability under red light depended mainly on the N-PAS and partly on the GAF domains. Hence, requirement of the *phyA* structure for each of the *phyA*-specific properties differed, suggesting that each domain of *phyA* has evolved coordinately to build *phyA*.

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P4.9

16:10 Tuesday 30th June 2009

The bHLH transcription factor SPT and DELLA proteins act together to regulate cell size of Arabidopsis cotyledons in a GA-dependant manner

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Most transcription factors from the basic-helix-loop-helix family XV have been characterised as a key component in phytochrome signalling in plants (Toledo-Ortiz et al., 2003). Amongst them, PIF1, PIF3, PIF4, PIF5, PIF6 and PIF7 have been shown to directly interact with the phytochrome molecule (Bae and Choi, 2008). PIFs also provide links to the hormones pathway. Indeed, it was recently shown that both PIF3 and PIF4 interact with GA-dependant growth repressor DELLA proteins to modulate hypocotyl elongation (Feng et al., 2008; de Lucas et al., 2008).

We have previously shown that SPATULA (SPT), a non phytochrome-binding PIF3 homologue, is a light-stable repressor of germination, that participates in integrating light and temperature signals to control seed dormancy, through the regulation of GA production (Penfield et al., 2005). Additionally, we showed that SPT action is not restricted to germination but, like PIF3 and PIF4, that it also controls seedling development.

Here, we describe the mechanism of action of SPT in the regulation of cotyledon expansion during seedling de-etiolation. As well as examining SPT targets, we will show that SPT acts in an integrative manner, together with other protein partners, to control cell expansion. A dual function for SPT, both as a transcription factor and as a protein stability regulator, will be discussed.

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P4.10

16:30 Tuesday 30th June 2009

FHY3 and FAR1 mediate red light input to the *Arabidopsis* circadian clock

Paul F. Devlin (Royal Holloway University of London), Hamad Siddiqui (Royal Holloway University of London)

The circadian clock is tightly tied to the light environment. Transcriptional feedback loops are able to generate a self-sustaining rhythm of approximately 24 h which impinges on almost every aspect of physiology in higher organisms. However, light signals are essential to maintain an exact 24 h rhythm.

In the model plant, *Arabidopsis thaliana*, an endogenous circadian rhythm is generated by a set of interlocked transcriptional feedback loops. Light directly affects the level of a number of the clock components in plants. The photoreceptors involved have been well characterised but the way in which they affect clock components is only beginning to be understood.

The transcription factors, FHY3 and FAR1, play a key role in red light input to the clock. We have shown that FHY3 and FAR1 positively regulate transcription of key clock components in red light. As a result, *fhy3* and *far1* mutant seedlings specifically display aberrant circadian rhythmicity under these conditions. Moreover, this specific action of FHY3 and FAR1 has revealed novel interactions between the various clock loops and has given us new insights into the mechanism by which light can fine-tune the clock throughout the cycle of day and night.

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P4.11

Poster Session – Tuesday 30th June 2009

Pseudostem artificial extension with colored tubes led to the modulation of leaf elongation in Tall Fescue (*Festuca arundinacea* S.)

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Leaf length is a key parameter in grass plant morphogenesis, and thus in the determination of grasslands agricultural use-value. Regarding leaf elongation regulation, the pseudostem appears to play a morphogenetic role, mainly via an influence on i) the timing of leaf tip emergence and ii) the length of the leaf growth zone. These effects have been demonstrated by incising or artificially extending

pseudostems, and are presumably due to light effects. In order to determine i) if no other physical factor than light interfered in these reactions, ii) if this putative light influence would be mediated by a qualitative or quantitative spectral modification, and iii) if sheath elongation is also dynamically impinged by the pseudostem length, we tested the effect of pseudostem extension with plastic tubes on the leaf growth of uncut tall fescue plants. Tubes exhibiting contrasted optical properties were used: red-colored tubes affecting the “blue” domain of the spectrum (cryptochrome stimulation), green-colored tubes affecting the Red:Far Red ratio (modification of the phytochrome equilibrium), transparent tubes and opaque foil tubes. It appeared that the less light can pass through the tubes the faster the leaves elongate, and the longer the leaves and the sheaths. Red and green tubes effects were not significantly different. These results support the hypothesis that the pseudostem morphogenetic effect is due to light effects. Furthermore, in this context, leaf elongation does not react to a qualitative modification of a unique domain of the light spectrum, but rather to a quantitative general decrease of the irradiance.

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P4.12

Poster Session – Tuesday 30th June 2009

Functional genomics approaches to study the involvement of transcription factors in the microalgae *Ostreococcus tauri* circadian clock

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The unicellular algae *Ostreococcus tauri* is known as the smallest free-living eukaryote. This photosynthetic organism contains a nucleus with a highly compacted genome, one mitochondria and one chloroplast. The whole genome was recently sequenced and allowed the annotation of numerous genes. Given that genes duplications are very rare in this organism, it represents a very interesting model because only few genes are suspected to be involved in key functions, particularly in the biological clock. The *O. tauri* clock mainly consists of positive and negative elements homologous to the ones known in *Arabidopsis thaliana* (TOC and CCA1), but more actors are likely to be involved, interacting in interconnected loops. We decided to focus on some transcription factors that are known to have an impact on the functioning of the circadian clock in several other organisms. We overexpressed transcription factor genes (with sense or antisense constructions) in *O. tauri* luciferase reporter lines. This allowed us to determine the effect of these genes on the rhythmical expression of a key clock protein by following the luminescence level of the line. We focused on transcription factors from the RRB (type-B response regulators) and bHLH (basic Helix-Loop-Helix) protein families, this latter being present as single member in *O. tauri* but widespread in animals, plants and fungi. Here, we report the preliminary results obtained for these two kinds of transcription factors.

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P4.13**Poster Session - Tuesday 30th June 2009****Blue light modifies the response of *Arabidopsis thaliana* to limiting nitrogen supply**

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Blue light influences plant architecture and morphogenesis and may therefore affect radiation interception efficiency. Nitrogen deficiency reduces shoot area and strongly impacts on plant growth. However, few studies have investigated the interactions between these two factors and their combined impact on plant growth. Such was the aim of this work.

Arabidopsis thaliana plants cultivated in growth chamber with the same level of PAR and of R:FR, were submitted to contrasted levels of blue light (2, 16, 26 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and nitrogen supply (3 and 10 mM nitrate). Six times throughout the vegetative growth, dimensions, dry mass, and nitrogen content of aerial organs (lamina, petiole and hypocotyl) were measured.

As expected, blue light limitation entailed an increase of organ length, whereas N deficiency led to a global decrease of plant growth and N content. Interestingly, an interaction was pointed out between blue light and nitrogen supply. If blue light deficiency did not quantitatively affect plant growth under optimal N supply, we found that it enhanced plant growth under N limitation. In other words, blue light shortcoming increased the nitrogen use efficiency of N-limited plants. This was not due to higher radiation use efficiency but to a large increase in leaf area and N quantity in low-blue \times low-N plants.

Beyond the practical interest of these results to avoid phenotypic artifacts in blue-photomorphogenesis or N-starvation studies, the better understanding of plant reactions to N and blue light co-deficiency may have interesting applications to the ecology of shaded vs. sun-lighted plants.

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