Journal of Photoscience (2002), Vol. 9(2), pp. 94-97

Regulation of Plant Growth by Light-Growth Hormone Interactions

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Light is one of the most important environmental factors that influence plant growth and development. It does not function independently but exerts its role through coordinated interactions with intrinsic developmental programs, such as hormonal regulation. One typical example is hypocotyl growth in which light signals are modulated through growth hormones. However, the underlying molecular mechanisms are largely unknown. We demonstrated that brassinosteroids play an important role in the light signal transduction in etiolated hypocotyl growth. A light-responsive Ras-like G-protein, Pra2 from pea, physically and functionally interacts with a cytochrome P450 that specifically catalyzes C-2 hydroxylation in brassinosteroid biosynthesis. The cytochrome P450 expression, along with Pra2, is induced in the dark and predominantly localized in the rapidly elongating zone of etiolated pea epicotyls. Transgenic plants with a reduced level of Pra2 exhibit a dark-specific dwarfism, which is completely rescued by brassinosteroid application. On the contrary, overexpression of the cytochrome P450 results in enhanced hypocotyl growth even in the light, which phenocopies the etiolated hypocotyl growth. It is therefore envisioned that Pra2 is a molecular switch that mediates the crosstalk between light and brassinosteroids in the etiolation process.

Key words : brassinosteroid, cytochrome P450, etiolation, G-protein, hypocotyl (epicotyl), light

INTRODUCTION

Plants constantly monitor the intensity, wavelength, direction, and duration of environmental light, and the perceived light signals are subsequently integrated into diverse growth and developmental processes throughout the whole life span, from seed germination to flowering, to achieve optimized growth under a given light condition. Many morphological studies suggest that growth hormones are also involved in the light-regulated plant growth and development [1,2]. For example, transgenic plants overexpressing phytochrome A, a red/far-red light absorbing photoreceptor, exhibit a dwarfish appearance as observed in mutant plants that are deficient in brassinosteroid (BR) or gibberellic acid (GA) biosynthesis and/or perception. It is also well established that auxin exerts a critical role in phototropism, a pivotal light response that ensures optimal perception of sun light by leaves for efficient photosynthesis. However, no direct molecular mechanisms have been demonstrated other than morphological evidences in most cases.

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Here, we present molecular, genetic, and biochemical data, demonstrating that light regulates plant etiolation by modulating BR biosynthesis in hypocotyls. A Ras-like G-protein mediates light signals into the BR biosynthetic pathway in the dark to stimulate hypocotyl elongation.

MATERIALS AND METHODS

Yeast two-hybrid screening. Yeast two-hybrid screening was carried out using the MATCHMAKER Two-Hybrid System (Clontech, Palo Alto, CA). The *pra2* gene was used as bait. A pea cDNA library used was constructed from 6 day-old dark-grown seedlings.

Expression of recombinant proteins. The *pra2* gene was cloned into the pGEX-4T-2 vector (Amersham-Pharmacia, Buckinghamshire, UK) and expressed in *E. coli* strain BL21. The cytochrome P450 was expressed via the intein-based expression vector pTYB2 (NEB, Beverly, MA).

Complementation with BRs. BR feeding experiments on *Arabidopsis* plants were carried out as previously described using various BR intermediates [3].

Cytochrome P450 activity assays. Thirty μg of yeast microsomal fraction and 5 μg of the recombinant cytochrome P450 protein were routinely used for each reaction. Five μg of the Pra2 protein was included whenever required.

RESULTS AND DISCUSSION

Pra2 is a Ras-like small molecular weight G-protein from pea [4]. We were interested in the physiological role of Pra2 since it is induced specifically in the dark and has been suggested to have a role in the phytochrome-mediated light signal transduction during hypocotyl growth [4,5]. A yeast two-hybrid screening was employed to identify functional target protein(s) that interacts with Pra2. We isolated a cDNA clone encoding a polypeptide of 495 residues with an estimated molecular mass of 34.7 kDa. It exhibits all the conserved motifs among different cytochrome P450 proteins with a relatively high sequence identity to those involved in GA and BR biosynthesis, such as DWF4 and CPD in plants [6]. The cytochrome P450 gene is expressed predominantly in the dark. We therefore named it DDWF1 for dark-specific DWF-like protein 1 (see below). Both Pra2 and DDWF1 were colocalized on ER membrane as judged by fluorescent fusion technology (data not shown).

To investigate the functional role of the Pra2-DDWF1 interaction, the *pra2* gene was introduced into tobacco plants. The light-grown transgenic plants were indistinguishable from control plants. However, significant phenotypic changes were observed in the dark. The dark-grown anti-sense transgenic plants exhibited short hypocotyls, which mimic the dwarfish hypocotyls of BR- or GA-deficient plants (Fig. 1). The sense transgenic plants also exhibited short hypocotyls in the dark due to a cosuppression as verified by analysis of the transcripts of the transgenes. These observations indicate that the short hypocotyls observed in the transgenic plants are correlated with the suppression of *pra2* gene or its homologue.

Based on the specific interaction of Pra2 with DDWF1, an enzyme homologous to those involved in BR and GA biosynthesis, we reasoned that the dark-specific short hypocotyls of the *pra2* transgenic plants might be due to reduced levels of endogenous BR and/or GA. The anti-sense transgenic plants were grown in the presence of various phytohormones, including brassinolide (BL), GA, auxin, cytokinin, abscisic acid, and salicylic acid. Among

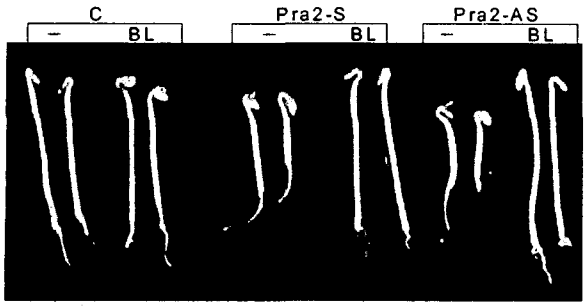

Figure 1. Transgenic tobacco plants with *pra2* gene grown in the dark. Plants were grown in the presence (BL) or absence (-) of brassinolide, a most BR hormon. C; control plant, Pra2-S; sense plants, Pra2-AS; anti-sense plants. The transcript levels of the *pra2* transgene were greatly suppressed in both the sense (Pra2-S) and anti-sense (Pra2-AS) plants due to a cosuppression event. [Adopted from Kang et al., Cell 105, 625-636].

them, only BL completely rescued the short hypocotyls (Fig. 1). These observations suggest that the anti-sense suppression of the Pra2 homologue in tobacco plants reduces BR biosynthesis by down-regulating the DDWF1-like activity. However, the *pra2* transgenic plants were unique from the known BR- or GA-deficient plants in that the short hypocotyls were observed only in the dark.

The *pra2* transgenic plants were fed with various BR intermediates to determine the biosynthetic step(s) regulated by Pra2. The castasterone (CS) and BL but not typhasterol (TY) completely rescued the short hypocotyls. The 6α-hydroxyCS and 6-deoxoCS also showed some restoring effects, about 50 - 60% of the BL effect, indicating that Pra2 regulates the DDWF1 activity that catalyzes the C-2 hydroxylations in BR biosynthesis [7].

Interestingly, anti-sense *ddwf1* transgenic plants also exhibited short hypocotyls in the dark, which were also rescued by BL and CS but not by TY as in the *pra2* transgenic plants. It is thus obvious that DDWF1 catalyzes C-2 hydroxylation steps in BR biosynthesis. It was of particular interest that the *ddwf1* sense transgenic plants exhibited elongated hypocotyls even in the light (Fig. 2). This phenotype resembles the etiolated hypocotyl growth, indicating that a primary role of DDWF1 is to induce hypocotyl elongation through up-regulation of BR biosynthesis in the dark, supporting a role for BR in hypocotyl growth.

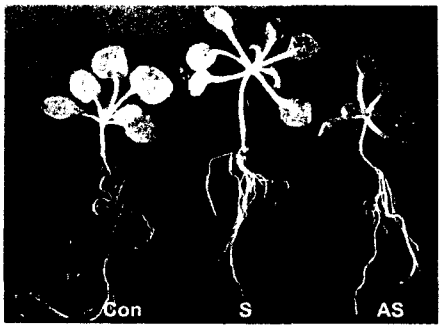

Figure 2. *ddwf1* transgenic *Arabidopsis* plants grown in the light. Con; control plant, S; sense plant, AS; anti-sense plant. Note that the hypocotyl of the sense plant is elongated like etiolated ones. [Adopted from Kang et al., Cell 105, 625-636].

Taken all together, our results strongly suggest that Pra2 is a molecular mediator that regulates DDWF1 in BR biosynthesis and integrates light and BR signals in the etiolated hypocotyl growth (Fig. 3).

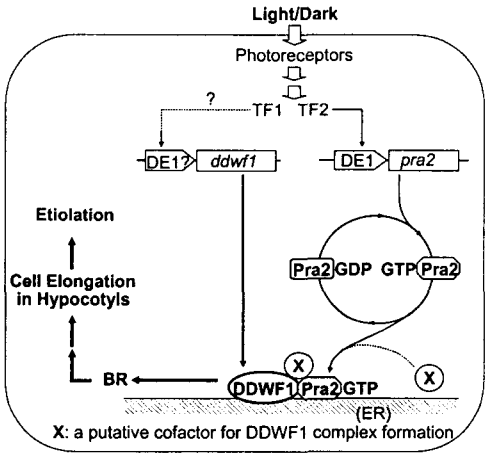

Figure 3. A working model for the Pra2-DDWF1 interaction. Both Pra2 and DDWF1 are localized on ER membrane. The full enzymatic activity of DDWF1 may require a hypocotyl-specific cofactor (X). GTP-GDP cycle may be an additional mechanism for regulation of BR biosynthesis. [Adopted from Kang et al., Cell 105, 625-636].

CONCLUSION

It is now evident that environmental light signals perceived by plants are further modulated by intrinsic developmental factors, such as growth regulators, as well as by other environmental cues. Diverse crosstalks among different signaling cascades were observed in various photomorphogenic processes, including etiolation and flowering time control, in which light, temperature and GA signals are genetically linked. It is therefore proposed that light signal transduction mechanisms in plants should be considered as a concept of signaling networks rather than signaling cascades (or signaling pathways). Furthermore, interactions and integrations among various signaling mediators, rather than function of each signaling mediator, should be more seriously considered.

REFERENCES

1. Neff, M.M., S.M. Nguyen, E.J. Malancharuvil, S. Fujioka, T. Noguchi, H. Seto, M. Tsubuki, T. Honda, S. Takatsuto, S. Yoshida and J. Chory (1999) *BAS1*: A gene regulating brassinosteroid levels and light responsiveness in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **96**, 15316-15323.
2. von Arnim, A.G. and X.-W. Deng, (1996) Light control of seedling development. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 215-243.
3. Fujioka, S., J. Li, Y.H. Choi, H. Seto, S. Takatsuto, T. Noguchi, T. Watanabe, H. Kuriyama, T. Yokota, J. Chory and A. Sakurai (1997) The *Arabidopsis deetiolated2* mutant is blocked early in brassinosteroid biosynthesis. *Plant Cell* **9**, 1951-1962.
4. Yoshida, K., Y. Nagano, N. Murai and Y. Sasaki (1993) Phytochrome-regulated expression of the genes encoding the small GTP-binding proteins in peas. *Proc. Natl. Acad. Sci. USA* **90**, 6636-6640.
5. Nagano, Y., Y. Okada, H. Narita, Y. Asaka and Y. Sasaki (1995) Location of light-repressible, small GTP-binding protein of the YPT/Rab family in the growing zone of etiolated pea stems. *Proc. Natl. Acad. Sci. USA* **92**, 6314-6318.
6. Szekeres, M., K. Németh, Z. Koncz-Kálmán, J. Mathur, A. Kauschmann, T. Altmann, G.P. Rédei, F. Nagy, J. Schell and C. Koncz (1996) Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and deetiolation in *Arabidopsis*. *Cell* **85**, 171-182.
7. Bishop, G.J., T. Nomura, T. Yokota, K. Harrison, T. Noguchi, S. Fujioka, S. Takatsuto, D.G. Jones and Y. Kamiya (1999) The tomato DWARF enzyme catalyzes C-6 oxidation in brassinosteroid biosynthesis. *Proc. Natl. Acad. Sci. USA* **96**, 1761-1766.
8. Blázquez, M., M. Koornneef and J. Putterill (2001) Flowering on time: genes that regulate the floral transition: Workshop on the molecular basis of flowering time control. *EMBO Rep.* **2**, 1078-1082.