Short Communication

Phototropin-Dependent Weak and Strong Light Responses in the Determination of Branch Position in the Moss Physcomitrella patens

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Branch position in the moss Physcomitrella patens is regulated by blue light. In this study, fluence rate dependency of branch position determination was investigated by partial cell irradiation with a microbeam. With a 30 W m⁻² or lower fluence rate, branches formed at the microbeam area, but formed outside the microbeam when the fluence rate was raised to ≥200 W m⁻². Thus, both weak and strong light responses influence the determination of branch position. Further, light sensitivity of both responses was reduced in phototropin knock-out lines, revealing an involvement of phototropin as the blue light receptor.

Keywords: Branch formation — Cryptochrome — Photomorphogenesis — Phototropin — Physcomitrella patens — Polarity.

Abbreviations: SLR, strong light response; WLR, weak light response.

Because of their sessile nature, plants have developed various mechanisms to adjust to the environment. Light is one of the most important environmental stimuli, and plants have evolved many photosensory pigments, including red/far-red photoreversible phytochrome, blue-light absorbing phototropin and cryptochrome (Glyula et al. 2003). Many photomorphogenic phenomena in vascular plants, such as seed germination, phototropism of hypocotyls or roots and floral induction, are regulated by these photoreceptors. Such phenomena are photoresponses at the tissue or organ level. However, photomorphogenesis is also observable at the cellular level in lower plants. Photoinduction of branch formation in the moss Physcomitrella patens is a good example of photomorphogenesis at the cell level (Imaizumi et al. 2002, Uenaka et al. 2005). Protonemata in P. patens are well suited for physiological analyses of cellular level photomorphogenesis because of their simple structure (Dyer and Duckett 1984). After spore germination, a single moss protonemal cell develops into a well-branched, multicellular protonemal colony under light conditions, which then forms gametophores (Reski 1997, Schaefer and Zryd 2001). Two cryptochrome genes, Pp CRY1a and Pp CRY1b, have been shown to be responsible for the blue light induction of branch formation (Imaizumi et al. 2002). Upon detailed physiological analyses, the branch induction via cryptochromes was enhanced by a red light receptor in or around the nucleus (Uenaka et al. 2005). Branch position was also regulated by light, and branches appear within the area of light irradiation when a single protonemal cell is partially irradiated with microbeam blue light (Uenaka et al. 2005). Depending on the light fluence rate, opposite responses, such as the positive and negative responses in phototropism, are frequently observed. In fact, negative and positive phototropism and polarotropism of protonemata were shown in P. patens (Jenkins and Cove 1983). However, blue light fluence rate effects on the determination of the branch position have not been reported. To elucidate the photo-regulatory processes in moss development, the positions of branch formation under microbeam blue light irradiation with various fluence rates were analyzed.

When whole second cells of a red light-grown protonema were irradiated with blue light, a branch appeared in the apical end of the cell regardless of the blue light fluence rate (Fig. 1A–D), indicating internal cell polarity. However, the branch appeared within the microbeam area when the cell was partially irradiated with microbeam blue light of 10 and 30 W m⁻² (Fig. 1E, F and Supplementary Movie 1). In contrast, microbeam irradiation with 200 and 400 W m⁻² blue light induced a branch not within the microbeam area, but outside the area (Fig. 1G, H and Supplementary Movie 2). The positioning is clearly different from that under whole-cell irradiation, because there is a distance between the branch and the apical septum. Hence, the positioning of branches outside the area of the microbeam is not merely the result of a disappearance of photoregulation under strong blue light irradiation, but
also because branch formation is regulated so as to avoid the area of strong light. Branch formation outside the area of strong blue light and within the weak blue light area was therefore termed the strong light response (SLR) and weak light response (WLR), respectively, in the light determination of branch position. Interestingly, branches always appeared at the apical, not the basal side of the microbeam in the SLR (Fig. 1I), suggesting the contribution of internal cell polarity. When the WLR was induced by partial irradiation with a larger microbeam (95 μm in diameter), branches appeared within the microbeam area, but the positions inside the area were biased towards the apical area (Fig. 1J). Thus, branch position is determined by blue light under the influence of internal cell polarity in both the WLR and SLR.

Four phototropin genes, *Pp PHOTA1*, *Pp PHOTA2*, *Pp PHOTB1* and *Pp PHOTB2*, have been identified in *P. patens*, and phototropin involvement in the WLR of branch position determination has been demonstrated using phototropin disruptants (Uenaka et al. 2005). Phototropin could also be a receptor for the SLR. Hence, the positions of branches induced under a blue microbeam of various fluence rates were studied in two lines of a triple disruptant of phototropin (*photA2photB1photB2-1* and *photA2photB1photB2-2*) (Kasahara et al. 2004). Branches formed within the microbeam area in wild-type cells irradiated with blue light of 10 Wm⁻² (Fig. 1E), but appeared at the apical end of the cell in disruptant cells, showing the lack of WLR at this intensity (Fig. 2A, B and Table 1). A WLR in disruptant cells was still induced when blue light of 30 Wm⁻² was used for irradiation (Fig. 2C, D and Table 1). When disruptant cells were irradiated with a blue light microbeam of 200 Wm⁻², the SLR did not occur, but the WLR did (Fig. 2E, F and Table 1). Nevertheless, when the fluence rate of blue light was raised to 400 Wm⁻², the SLR was induced in the disruptant cells (Fig. 2G, H and Table 1). These results indicate that the blue light sensitivity of both the SLR and
WLR is reduced in phototropin triple disruptant lines and, therefore, both the SLR and WLR are regulated through phototropin. At least one of the disrupted phototropins, photA2, photB1 or photB2, is responsible for the WLR and for the SLR. Further, as the SLR and WLR are still observable in the triple disruptant lines, the non-disrupted phototropin, photA1, may also mediate both the WLR and SLR.

In this study, blue light was shown to induce the SLR as well as the WLR in the determination of branch position. The SLR was also found to depend on phototropins, as is the case for the WLR. Further, detailed examination revealed that cell polarity contributes to the positioning of branches in both the WLR and SLR. Thus, the processes of branch formation in _Physcomitrella patens_ can be summarized as indicated in Fig. 3: after the photoinduction of branches through cryptochromes, signals from both phototropin and cell polarity determine the position of new branches in _P. patens._
Materials and Methods

Plant materials and aseptic culture

As stock cultures, the wild type and two lines of phototropin triple gene disruptants (photA2photB1photB2-1 and photA2photB1photB2-2) of P. patens were grown under white light emanating from a fluorescent tube (FL20SD; Toshiba Lighting Technology Corp., Tokyo, Japan). BCDAT medium was used for propagation and for physiological experiments (Nishiyama et al. 2000). For experiments, protonemata were cultured for 5 d under unilateral red light of approximately 0.7 Wm\(^{-2}\). The protonemata were rarely branched and the cells had the mixed features of a chloronema and a caulonema, i.e. they had many chloroplasts (typical of a chloronemal cell) and the cross-walls were sometimes perpendicular (typical of a chloronema) and sometimes oblique to the protonemal axis (typical of a caulonema) (Kadota et al. 2000). All procedures were performed at 25\(^{\circ}\)C.

Microbeam irradiation

Microbeam irradiation was performed on a custom-made microbeam irradiator as described previously (Kadota et al. 2000). Monochromatic blue light was provided through an interference filter (Optical Coatings Japan, Tokyo, Japan), which had its peak at 450.0 nm and a half-band width of 36 nm. Neutral density filters (Inconel-coated quartz glasses, Fujitoku Crop., Tokyo, Japan) were used to attenuate the fluence rate. The second cells of the red light-grown protonema were irradiated for 1 d with microbeam blue light of 40 or 95 \(\mu\)m in diameter.

Supplementary data

Supplementary data are available at PCP Online.

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References


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