

## **Ca<sup>2+</sup>-Regulation of Phototropism and Blue-Light-Induced Branch Formation in the Coenocytic Stramenopile Alga, *Vaucheria*.**

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A tip-growing coenocytic Stramenopile (Chromista, Xanthophyta), *Vaucheria*, is a suitable research material for blue light (BL) responses because it apparently lacks phytochrome system. *Vaucheria* has a unique ability to change the direction of phototropic bending towards or away from unilateral BL in response to light intensity. Although positive phototropism of this alga have been analyzed in some detail, studies of negative phototropism has long been impeded because of the requirement for a strong BL source and a long irradiation time required. Developing new methods we analyzed the light-intensity-dependent transition from positive to negative phototropism and hypothesized that a BL-induced influx of Ca<sup>2+</sup> at the apex and eventual local transient rise of cytoplasmic Ca<sup>2+</sup> level mediates the positive to negative phototropic inversion. In Part I, Ca-regulation of phototropism will be dealt with, and in Part II, I will present some recent findings on BL-induced branch initiation from the irradiated locus.

### *Part I. Ca-regulation of Phototropism.*

The alga's positive phototropic bending was not influenced by simultaneously given, strong red background light. If, however, the alga was illuminated with blue or green background light in a solution supplemented with 1-4 mM Ca<sup>2+</sup>, simultaneously with the unilateral BL, curvature towards unilateral BL source greatly decreased and finally arose a negative curvature. Magnitude of the negative curvature was the function of the product of Ca<sup>2+</sup> concentration and fluence rate of the background blue/green light. Addition of Ca<sup>2+</sup> channel blockers (La<sup>3+</sup>, verapamil, nifedipine and nitrendipine) inhibited the phototropic inversion. By contrast, A23187 (plus 4.4mM Ca<sup>2+</sup>) mimicked the effect of background blue/green light; i.e., it caused negative curvature under safe red background light.

Although the simultaneous background illumination method provides a good simulation of the negative phototropism observed in natural sunny habitats, use of an intense unilateral BL source alone is far better for kinetic analysis of the negative phototropism. Without any supporting background light (in the presence of 4.4mM Ca<sup>2+</sup>), a unilateral 457.9 nm pulse (>500Wm<sup>-2</sup>, >10 s) from a CW argon-ion laser could induce negative bending. The phototropic inversion was sensitized by 4.4mM Ca<sup>2+</sup>. Positive phototropic response before reaching its maximum in the  $\Lambda$ -shaped fluence-response curves followed reciprocity law and was not influenced by Ca<sup>2+</sup> concentration. In the decreasing arm of the fluence-response curves reciprocity law did not hold. The results not only demonstrated that negative phototropic bending does not require long irradiation time if the intensity is sufficiently high but indicates that the alga senses the fluence rate of the unilateral BL and regulates the direction and size of the phototropic response 10-100s after the onset of light. The study also suggests the possibility that positive and negative phototropism might be mediated by two separate photosystems.