Action spectra of the light-growth response in three behavioral mutants of Phycomyces*

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Abstract. Null-point action spectra of the light-growth response were measured for three mutants of Phycomyces blakesleeanus (Burgeff) and compared with the action spectrum of the wild type (WT). The action spectrum for L150, a recently isolated “night-blind” mutant, differs from the WT spectrum. The L150 action spectrum has a depression near 450 nm and small alterations in its long-wavelength cutoff, the same spectral regions where its photogravitropism action spectrum is altered. This indicates that the affected gene product influences both phototropism and the light-growth response. For L85, a “hypertropic” (madH) mutant, the light-growth-response action spectrum is very similar to that of WT even though the photogravitropism action spectrum of L85 has been shown previously to be altered in the near-UV region. The affected gene product in this mutant appears to affect phototropic transduction but not light-growth-response transduction. The action spectrum of C110, a “stiff” (madE) mutant, differs significantly from the WT spectrum near 500 nm, the same spectral region where sporangiophores of madE mutants have been shown to have small alterations in second-derivative absorption spectra. This indicates that the madE gene product may be physically associated with a photoreceptor complex, as predicted by system-analysis studies.

Key words: Action spectra – Blue light – Light-growth response – Phycomyces – Sporangiophore

Introduction

The light-growth responses of Phycomyces wild type (WT) and behavioral mutants have been compared previously using classical step-up or step-down light stimuli (Galland and Lipson 1987). These studies demonstrated that a mutant’s defect in phototropism is usually accompanied by an analogous defect in the light-growth response. In particular, each of the “night-blind” mutants (defective in genes madA, madB, and madC) exhibits a similar shift in absolute threshold and increase in latency for both phototropism and the light-growth response (Foster and Lipson 1973; Bergman et al. 1973). “Stiff” mutants (defective in genes madD through madG) all have reduced light-growth-response amplitudes (Foster and Lipson 1973) analogous with their reduced phototropic curvatures (Bergman et al. 1973).

The light-growth response has also been studied by system-analysis methods employing Gaussian white noise and “sum-of-sinusoids” test stimuli. Analyses of single and double mutants indicated that the eight mad-gene products (MadA through MadH) function as molecular components of a photosensory transduction complex (Poe et al. 1986; Palit and Lipson 1989; Palit et al. 1989). In particular, the mad-gene products classically considered (Bergman et al. 1973) to be near the growth-control output (MadD through MadG) appear to be closely associated with gene products assumed to be near the photoreceptor input (MadA, MadB, and MadC).

In several other studies, the mad mutants have been shown to differ from WT. Photogravitropism action-spectra are altered in madB, madC, and madH mutants (Galland and Lipson 1985) and in three strains of a new class of night-blind mutants (Alvarez et al. 1989; Ensminger et al. 1990; Campuzano et al. 1990). Following sequential ultraviolet (UV; specifically UV-A) and blue irradiation, the mycelium (Berns and Vaughn 1970) and sporangiophore extracts (Trad et al. 1988) of madC mutants exhibit a slower decay of light-induced absorbance changes. In addition, dark-grown sporangiophores of two madE mutants, but not madB or madC mutants, have altered second-derivative absorption spectra. It was suggested (Horwitz et al. 1986) that the absorbance differences detected in madE mutants might also appear in physiological action spectra.

In the present study, we have compared the light-growth-response action spectrum of WT with one representative strain from each of the three classes of beh...
havioral mutants. L150, a recently isolated "night-blind" mutant, and L85, a "hypertropic" (madH) mutant, were chosen to determine whether the alterations in their photogravitropism action-spectra (Galland and Lipson 1985; Ensminger et al. 1990) also appear in their light-growth-response action spectra. C110, a "stiff" (madE) mutant, was chosen to test the prediction that this mutant might have an altered action spectrum (see above; Horwitz et al. 1986).

Materials and methods

Strains and growth conditions. The mutants used in this study, L150 (Alvaraz et al. 1989), L85 (Lipson et al. 1983), and C110 (Bergman et al. 1973), were isolated from the WT strain NRRL1555 of Phycomyces blakesleeanus (Burgeff) after mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine. Growth conditions were the same as in the previous study (Ensminger et al. 1991) and sporangiophores from the second through fourth crops were used for all experiments.

Experimental methods. We used the "staircase stimulus protocol" to determine the effectiveness of each wavelength (test light) relative to a fixed-intensity (77 μW·m⁻²) 450-nm reference light, as described in detail by Ensminger et al. (1991).

Because the same 450-nm reference light was used for all action spectra (see above; Ensminger et al. 1991), the action spectrum of each mutant was compared with that of WT by superimposing them at 450 nm. We then calculated a difference spectrum by subtracting the log effectiveness of WT from the log effectiveness of the mutant. In the difference spectra, standard errors (SEs) were assigned to each point using the error-propagation relationship:

$$\sigma_d = \sqrt{\sigma_w^2 + \sigma_m^2}$$

where $\sigma_w$ is the SE for WT, $\sigma_m$ is the SE for the mutant, and $\sigma_d$ is the SE of the difference between WT and mutant spectra. To test for significant differences, two-tailed t-tests (Sokal and Rohlf 1981) were employed.

Results and discussion

Night-blind mutant, L150. The light-growth-response action spectrum of L150 differs from that of WT (Fig. 1). In particular, the difference spectrum (Fig. 1b) shows that the effectiveness of L150 is elevated by about 0.1 log unit from 383 to 431 nm and also deviates from WT above 450 nm. It should be emphasized that, except for the 450-nm point, these two action spectra are very similar in shape from 383 to 478 nm. In other words, the quantum effectiveness of L150 appears to be depressed near 450 nm and shows as light trend for increasing effectiveness as a function of wavelength above 450 nm (Fig. 1b).

Because 450-nm reference light was used to determine this action spectrum, the question arises as to whether the suppression at this wavelength could be an artifact. This seems unlikely because the photogravitropism action-spectrum of L150 (Ensminger et al. 1990), determined at threshold intensities without any reference light, also exhibits a suppression near 450 nm and has an altered long-wavelength cutoff.

Thus, the alterations in the light-growth-response action spectrum of L150 (Fig. 1) are not restricted to the

![Fig. 1a, b. Null-point action spectra and difference spectrum for the light-growth response of mutant L150 and WT Phycomyces. a Action spectra of mutant L150 (closed circles) and WT (open circles). In this figure and in Figs. 2a and 3a, log relative effectiveness is shown as a function of wavelength and the two action spectra were superimposed at 450 nm. Where error bars are not shown, they are smaller than the size of the datum point. b Difference spectrum. In this figure and in Figs. 2b and 3b, log relative effectiveness" of the mutant minus "log relative effectiveness" of WT is shown as a function of wavelength. For SE calculation method, see Materials and methods](image-url)

![Fig. 2a, b. Null-point action spectra and difference spectrum for the light-growth response of mutant L85 (closed circles) and WT (open circles) Phycomyces. See Fig. 1](image-url)

![Fig. 3a, b. Null-point action spectra and difference spectrum for the light-growth response of mutant C110 (closed circles) and WT (open circles) Phycomyces. See Fig. 1](image-url)