

West, D. J. Phase shift of circadian rhythm of conidiation in response to ultraviolet light.

Light-induced advances and/or delays in phase are fundamental responses of circadian rhythms. Determination of the qualities of light that are most effective may help identify the photoreceptor(s) mediating the phase shift and may be useful in establishing the biochemical composition of the oscillator generating the observed rhythm.

Sargent and Briggs (1967 *Plant Physiol.* 42: 1504) examined the effects of light from the visible and near ultraviolet portions of the spectrum on a circadian rhythm of conidiation in growth tube cultures of *N. crassa*. Extended exposure to low intensities of light in the near ultraviolet or blue regions of the spectrum proved especially effective in suppressing expression of the conidiation rhythm. The form of the action spectrum derived from this study suggested a carotenoid or a flavin compound as the primary photoreceptor. Brief exposures to white light at various times during the conidiation cycle did not suppress the rhythm but did result in phase advances or delays. It was assumed that the components of white light responsible for the phase shifts were the same as those that suppressed the rhythm on extended exposure.

I have now examined the effect of short wavelength ultraviolet light as a phase shifting agent for the circadian rhythm of conidiation in *N. crassa*. Growth tubes were constructed of 12 mm i.d. Suprasil T21 quartz tubing (Amersil Inc., Hillside, N.J.). This type of quartz was necessary to avoid a blue fluorescence that occurred when cheaper grades of quartz tubing were irradiated with ultraviolet light. The tubes were half filled with a medium consisting of Vogel's minimal N salts plus 0.3% glucose, 0.5% arginine-HCl and 1.5% agar. Growth tubes were inoculated with conidio of the bd strain (mating type A, no isolation #, FGSC #1858; obtained from M. L. Sargent) and were incubated at 25° C for one day under 1100 lux of light from cool white fluorescent bulbs. They were then placed in continuous darkness except for indirect illumination from a red safelight (G.E. model BCJ, 60 watt) which did not affect the conidiation rhythm. Under these conditions a band of conidia was half formed at the growing front approximately 8-10 hours after the light-dark transition. The middle of subsequent conidiation bands occurred at approximately 22.5 hour intervals.

Individual growth tube cultures received a single 5 minute exposure to short wavelength ultraviolet light (total dose 810 ergs/mm²) at times ranging from 10-35 hours after the middle of the third conidiation band. Nearly pure 254 nm light was obtained from a Sylvania G15T8 genicidol lamp mounted behind a baffle containing a 1 x 1 inch interference filter. During exposure, the growth tubes were positioned so that an area extending from approximately 5 mm ahead to 20 mm behind the growing front was irradiated. Irradiated tubes were subsequently incubated in continuous darkness until the ninth conidiation band had formed. The growth fronts of both irradiated and unirradiated control tubes were marked at 4-6 hour intervals so as to bracket the middle of the third and

eighth conidiation bands. The tuber were later scanned with a densitometer coupled to a chart recorder. From these tracings and the previous growth front measurements, the elapsed time between the middle of the third and eighth conidiation bonds was calculated for each growth tube. If this interval for on irradiated tube was shorter or longer than the average interval from a set of un-irradiated control tubes, then the difference was recorded as a phase advance or delay respectively.

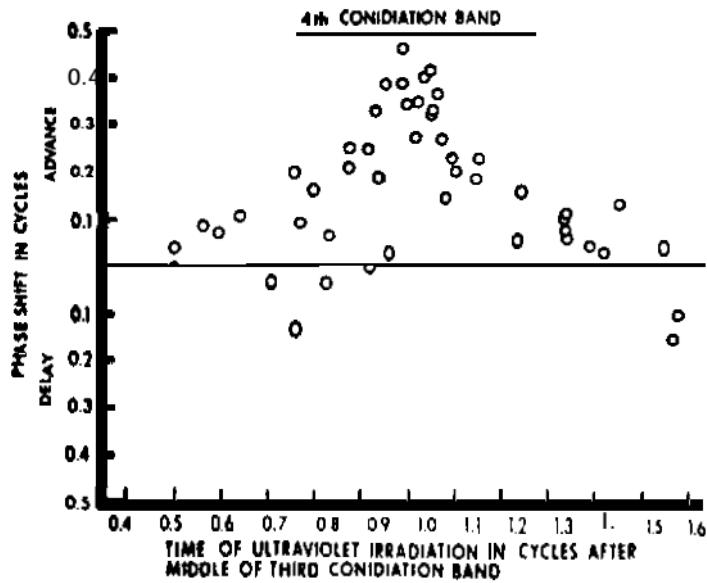


Figure 1 shows the combined data from nine independent experiments. Phase shifts are plotted as advance or delay in fractions of a cycle versus the stage at which the culture was irradiated. It is clear that maximum phase advances occurred when ultraviolet light was administered around the middle of the fourth conidiation bond. Lesser advances occurred when the light pulses fell during the early and late phases of conidiation. In general, only small phase shifts resulted when the tubes were irradiated during the nonconidiated or interband phases of growth. A very few instances of substantial phase delay were observed when irradiation occurred during late interband. These results are partially in accord with the pattern of phase shifts that Sargent and Briggs induced with white light. They also found maximum phase advances when white light exposures coincided with the middle of a conidiation bond. However, they did observe substantial phase delays with white light pulses administered throughout the late interband and early conidiation phases of growth.

The effectiveness of short wavelength ultraviolet light could indicate a role for nucleic acids in the phase shifting mechanism. In this context it would be of interest to determine whether phase shifts induced by ultraviolet light could be reversed by visible light. However, a rigorous

test of this possibility requires that there be phases in the cycle where ultraviolet light causes substantial phase shift while visible light does not. The rather close correspondence in the ultraviolet and white light induced phase shift profiles makes this test very difficult, if not impossible, to carry out. ■ ■ ■ Biology Division, Kansas State University, Manhattan, Kansas 66506.