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Light effects on circadian rhythm of non-conidioting N. crassa (bond)

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Abstract

Light effects on circadian rhythm

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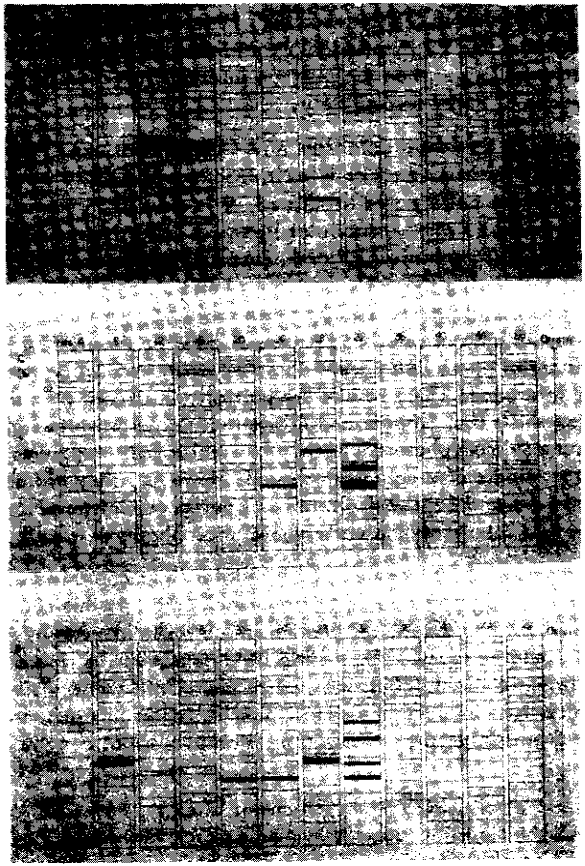
In various models it hos been proposed that circadian clocks effect photoperiodic time measurement by establishing a given phase relationship between the oscillation and the light cycle. Thus the light-dark, and the dark-light transition serve as entraining signals.

The band strain of N. crassa exhibits a circadian rhythm of conidiation when grown on a solid medium of appropriate composition (Sargent et al. 1966 Pl. Phys. 41: 1343). Hochb.rand Sargent (1974 J. Bacteriol. 120: 1164) have demonstrated an oscillation in the activity of several of the enzymes associated with conidiogenesis in this strain. We have sought a biochemical manifestation of the clock in the absence of any obvious morphological rhythm in cultures receiving either continuous light (LL), continuous darkness (DD) or a light-dark transition (LD).

The bond mutant (FGSC #1859) was cultured in a liquid medium containing 1.5% sucrose, 0.23% sodium acetate, 0.1 ml Tween 80/L and Vogel's salts. Under these conditions conidiation is inhibited (Siegel et al. 1968 Experientia 24: 1179). A series of 250 ml flasks each containing 50 ml of medium was inoculated with 0.2 ml of a conidial suspension and the flasks were divided into three groups; LL flasks were removed to a lighted growth chamber and received 250 footcandles of continuous light until harvested, LD flasks received 2 hours of illumination (250 footcandles), were wrapped in aluminum foil and removed to a darkened growth chamber until harvested. DD flasks were wrapped in aluminum foil and removed to a darkened growth chamber until harvested. All cultures were maintained at 25° C. The time scale for harvesting began 24 hours after inoculation and this interval was designated "germination period".



At 4 hour intervals, through 48 hours (beyond germination period), a flask was removed from each group to a darkroom and under illumination from a Kodok IA red safe light a crude extract was prepared for electrophoresis. The mycelial mats were dried on filter paper and quickly transferred to a chilled mortar. A slurry was prepared by grinding these mats with sand and 2 ml of cold 0.1 M Tris-glycine buffer pH 8.3. This slurry was centrifuged at low speed ond the supernatant fluid obtained was frozen until used for electrophoresis. Electrophoresis on 7% polyacrylamide gel was carried out using the method of Davis (1964 Ann. N.Y. Acad. Sci. 121: 404) ond the formulations of Canalco (1968 Canal Industrial Corporation, Rockville, MD). The proteins were stained with analine blue block.

Figure 1 A, B, C is a diagram of the electrophoretic patterns of the acidic proteins extracted from each of the cultures. The degree of similarity among replicate gels is high ond each diagram represents a composite of three replicate gels. A scale of Rf values is to the left of each set of gels. Similarities and differences among the gels receiving a given light treatment con be seen comparing the bonding patterns for cultures harvested at different times. Similarities and differences among the cultures receiving different light treatments con be seen by comparing the banding patters for cultures harvested at the some time.

Although some of the protein bands appear ubiquitous and others show unexplained variations, several major differences in bonding patterns can be seen. For example, at 32 hours DD and LD hove three identical darkly staining proteins, whereas LL has none. The sudden dramatic appearance of the protein band Rf 0.61 in LD culture at 8 hours ond 28 hours (Fig. 1B) is interesting ond suggests on extremely rapid synthesis and turnover. No similar pattern was detected in either of the other light treatments. Although this some band did not recur at 48 hours it moy be significant that the only place where this "oscillation" was observed was in the cultures receiving a light-dark tmnsition. We moy be observing a protein manifestation of the "clock" under conditions where where the morphological rhythm of conidiotion does not occur.

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