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A new culture method for biochemical studies of the circadian clock

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Abstract

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The band strain of N. crassa exhibits a well-characterized circadian rhythm of conidiation under conditions of constant temperature and darkness (Sargent et al., 1966 Plant Physiol. 41: 1343-1349). Biochemical and physiological studier of the circadian clock have been very difficult because most cultures used in such experiments have been grown on solid medium to observe the bonding pattern. We have now been able to show that homogeneous, non-bonding cultures also exhibit normal circadian rhythmicity. Included in our studies were lawn-inoculated cultures on solid medium, shaking liquid cultures, and standing liquid cultures. Methods and results ore presented only for the latter because that system has been characterized the most extensively and potentially may be of the most use.

Cultures were maintained on Horowitz complete slants. All other medium, both liquid and solid, was Vogel's salts containing 1.2% sodium acetate and 0.05% casamino acids [solid medium contained 1 .5% agar]. bd A conidia from 6-B day old slants were suspended in distilled water or (in later experiments) liquid medium, and filtered through glass wool. The concentration of conidia in the filtrate was measured (Klett-Summerson colorimeter, blue filter), and an aliquot immediately added to a large volume of stirring liquid medium to give a final concentration of 2x 10^5 spores/ml. Using on automatic pipetter, 25 ml of the stirring suspension were added to each of several dozen 100 x 15 mm plastic disposable Petri dishes. Six growth tuber with solid medium were inoculated at one end with about 50 microliters of undiluted filtrate. All plates and growth tubes were put in constant light at 25° C. After about a day, they were transferred to constant darkness in on environmental growth chamber, also at 25°C. The Petri dish cultures hod visible growth by 24 hours, and subsequently formed a mycelial mot which covered the surface. At several different times after the cultures were placed in the dark, six pieces of mycelium from each of three plates were cut with a cork borer (11 mm diam.) and transferred to fresh growth tuber. (The size of the transferred piecer has been varied greatly with identical results.) The growing fronts of control and experimental growth tuber were marked in red safelight (G. E. BCJ, 60 watt) at the same time each subsequent day. After about 7 days in the dark, the timer of occurrence, or phases, of the first conidial bands of the experimental growth tuber were determined by linear regression analysis and compared to the corresponding band of the control tuber.

The phases observed in the growth tubes inoculated with pieces of the standing liquid cultures were very close to those of the controls at all timer sampled. (In some experiments small and consistent phase advances were seen in the experimental tubes.) The sampling manipulations therefore da not affect the phase of the clock, and it may be concluded that the liquid cultures hove a normal circadian clock whore phase is ret, like that of the controls, by the light-to-dark transition. The phases of experimental growth tubes start to differ from that of the controls at approximately the time when the liquid cultures reach stationary phase (about 55-60 hours of age). It seems likely that either the clock of older cultures "runs down," or that older cultures are susceptible to phase resetting when cut and transferred. This appears to be largely independent of the length of time they spend in constant light. Preliminary experiments suggest that a 3-hour period in constant light (possibly even less) suffices to set the phase of these cultures.

Experiments are underway to relate the age of the cultures and nuclear division timer to the functioning of the clock. Other types of experiments, particularly the addition of various agents to determine their effects on the clock, ore now possible.

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