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Abstract

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Summary Phase shifts of 4-5 h in the conidiation of bd, comparable to those caused by white light (4.0 J/m^2 sec), were detected by giving a pulse of a gibberellin, (gibberellic acid) an auxin, (2,4-dichlorophenoxyacetic acid) and a cytokinin, (kinetin) to cultures in liquid media and subsequently transferring the treated mycelia1 mat to solid media in race tubes. These plant growth regulators may affect the intracellular concentration of second messengers, including cyclic 3',5'-AMP.

Introduction It has been suggested that rhythmic conidiation in bd, cpd-1 and cpd-2 is induced by a rhythmic oscillation in the concentration of cyclic 3',5'-AMP (Hasunuma, K. Proc. Japan Acad. 60: Ser. B, 377-380, 1984). Reduced levels of cyclic 3'5'-AMP, resulting from the induction of orthophosphate regulated cyclic phosphodiesterase (cPDase) also lead to the formation of aerial hyphae and conidia (Hasunuma, K. Bot. Mag. Tokyo 98:203-217, 1985; Hasunuma, K. and Shinohara, Y. Curr. Genet. 10:197-203, 1985; Hasunuma K., Neurospora Newsl. 32:19-21, 1985). An external light signal causes a change in the concentration of cyclic 3'5'-AMP and -GMP (Hasunuma, K., Y. Shinohara, K. Funadera, and K. Furukawa, Chronobiologica in press, 1986).

Likewise in animals, several kinds of hormones, including adrenalin, function as first messengers (external signal) affecting the activity of adenylate cyclase or inositol phospholipid phosphodiesterase (Berridge, M.J. Biochem. J. 220:345-360), 1984). Thus it seems that some kinds of hormones may act as a zeitgeber, similar to light, affecting the intracellular concentration of cyclic 3'5'-AMP. We checked this possibility using the plant growth regulators gibberellic acid (GA3), kinetin (KIN) and 2,4-dicholorphenoxyacetic acid (2,4-D). The mode of action of plant growth regulators has remained unknown. One of the main reasons may be the lack of studies of cyclic 3’5’-AMP and -GMP. In this communication we describe a simple, accessible method to analyze the mode of action of hormones.

Experimental Conidia of strain bd a (FGSC #1859) grown for 5 days on glycerol complete slant media were suspended in sterilized water and filtered with gauze to remove mycelia. A conidial suspension (10^6 conidia/ml, 0.1 ml) was inoculated into 10 ml of Fries minimal -1.5% sucrose liquid media in several petri dishes (6 cm in diameter). These were incubated at 25°C in darkness for 12 h, irradiated with white light (3.3 J/m^2 sec) for 12 h, and then kept in darkness (free-running). The following procedures were carried out under a red safelight. Every 3 h after the onset of free-running conditions, 1 ml of Fries minimal medium (Fig. 1, Control), 1 ml of 5 x 10^-3 M GA3, 1 ml of 10^-4 M KIN, 1 ml of 10^-5 M 2,4-D or 1 ml of 5 x 10^-3 M acetic acid each in Fries minimal medium was added dropwise to the cultures, or else the cultures were directly irradiated with white light (4.0 J/m^2 sec). They were gently swirled, kept in darkness or in the light for 1 h at 25°C, and then washed three times with 20 ml of Fries minimal medium. The mycelia were taken, drained well with sterilized filter paper and inoculated at the end of race tubes (25 cm in run length) containing 10 ml of Vogel's salts, 1.2% Na acetate, 0.05% Casamino acids (Difco) and 2% agar.

Results and Discussion The results in Fig. 1 show that in the control experiment, the phase of the conidiation rhythm was changed by 5-6 h, implying that this procedure caused weak resetting of the underlying clock. These results are different from those using discs of mycelial pad (Perlman, J. and J.F. Feldman, Neurospora Newsl. 26:21, 1979), in which there was no change in the phase of the conidiation rhythm (Nakashima, H. Plant and Cell Physiol. 22:231-238 1981). Our method included no harmful treatment such as cutting out of mycelial discs, and also included differences in liquid and solid media. The differences may result in the observed phase shift. On Fries salts-1.5% sucrose-1.5% agar medium, however, bd produced very poor conidia, and the medium could not be used for the analysis of conidiation rhythm. Further, on Fries salts-0.5% sucrose-0.5% yeast extract (Difco)-2% agar medium, which supported conidiation, 9.6 h of phase advance of conidiation at CT 25.1 was observed by transferring mycelial mat from liquid medium to solid medium.

In each case, the phase change in the control (addition of Fries medium only) was subtracted from the phase change caused by the particular treatment. Light caused a 3.1 h phase delay at circadian time (CT) 15.3, and a 2.7 h phase delay at circadian time 25.1. GA3 caused a 3.8 h phase delay at CT 12, and a 5.0 h phase advance at CT 21.8.

KIN caused a 3.9 h phase advance at CT 21.8, with no obvious phase delay. 2,4-D gave a 3.8 h phase advance at CT 21.8-25.1 and a 2.9 h phase delay at CT 31.6. Although the effect of KIN on the phase shift of conidiation rhythm was not so great, the effects of GA3 and or 2,4-D were comparable to those of light (Fig.1) (Nakashima, H. Plant and Cell Physiol. 22:231-238, 1981). As another control experiment 5x10^-4 M of acetate was added to Fries liquid medium. Neither change in pH value of the medium (pH 6.0) nor change in the phase of conidiation was observed. These results support the finding that GA3 and 2,4-D promote the elongation of young hyphae of N. crassa (Tomita, K., T. Muryama, and T. Nakamura, Plant and Cell Physiol. 25:255-258, 1984). The effects of these plant growth factors on the intracellular concentrations of cyclic 3',5'-AMP and -GMP are required to be determined for the establishment of this method.

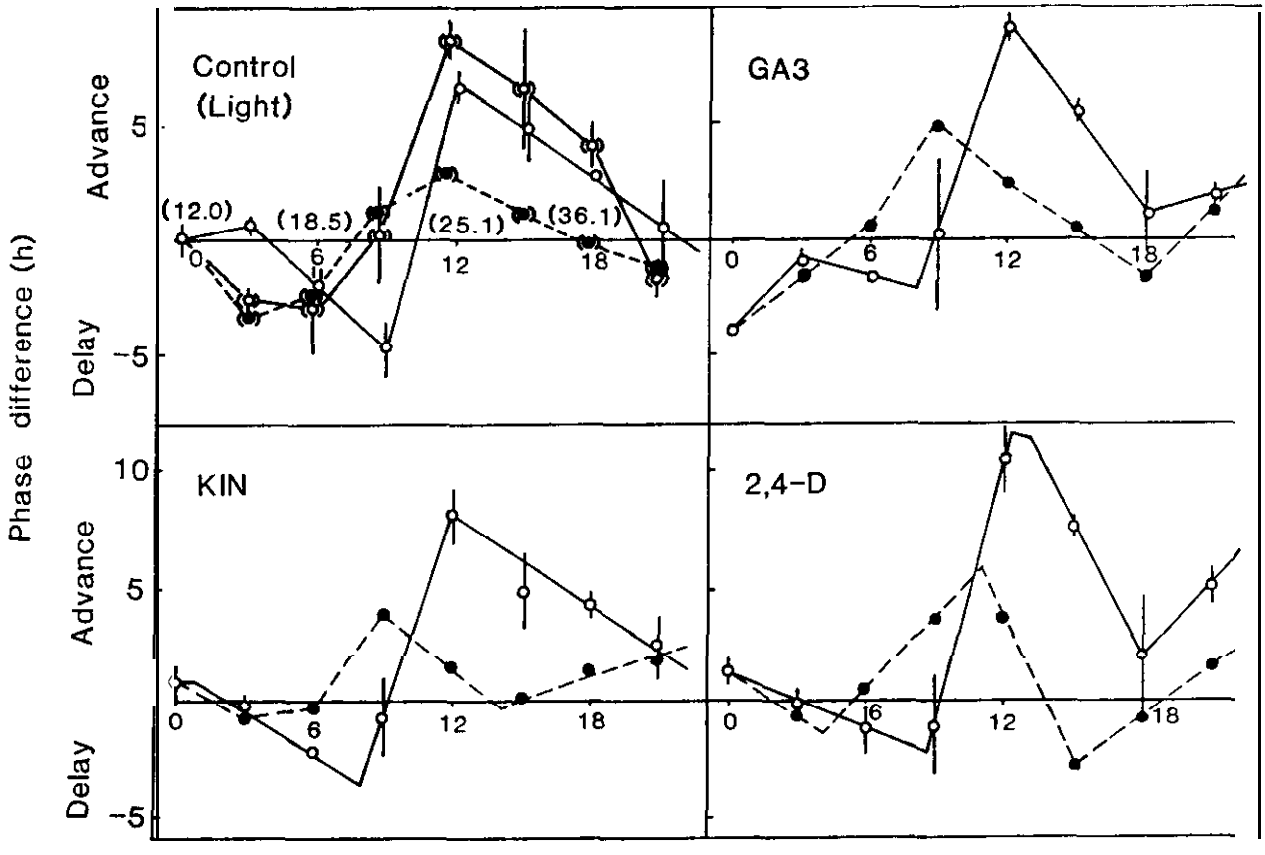

Fig.1. Phase response curves of conidiation rhythm of bd grown in liquid media and then transferred to solid media in race tubes, and those exposed to white light and to plant growth factor GA3, KIN or 2,4-D in liquid media. Mycelia of bd were exposed to white light (4.0 J/m^2 sec), 5x10^-4 M GA3, 1x10^-5 M KIN or 10^-6 M 2,4-D for 1 h at every 3 h after the onset of free running. The band formed in the control experiment at the onset of free running (CT 12) was used as a standard for the measurement of phase advances and delays. All the measurements were carried out in triplicate; the values shown are means with standard errors. Phase response curves ( ). Difference of phase change from control experiment ( ). In the case of white light irradiation ( ), an independent control experiment was carried out and differences in phase change from the control experiment are presented ( ).

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