Light-induced Encystment of *Blastocladiella emersonii* Zoospores

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SUMMARY

A low proportion of zoospores from dark-grown plants of *Blastocladiella emersonii* encyst if the suspension of zoospores is maintained in darkness, but visible light causes increased encystment. A lag in the response to light is eliminated by incubating spores in darkness prior to exposure. Light-induced encystment is insensitive to cycloheximide, but ceases when illumination ceases. Light has no effect on encystment of zoospores derived from light-grown plants; these will encyst even if kept in darkness.

INTRODUCTION

Light-stimulated growth, when defined in terms of increasing weight, is rare (Carlile, 1965, 1970) among fungi. The most adequately documented instances occur in aquatic fungi, the fresh-water Chytridiomycete *Blastocladiella emersonii* (Cantino & Horenstein, 1956*a*) and the chytrid-like marine Oomycete, *Thraustochytrium roseum* (Goldstein, 1963). With *B. emersonii*, visible light has a manifold effect (Cantino, 1965) upon growing thalli, and results in changes in dry weight, pools of small and large molecules (including a cytochrome-*b* type haemoprotein), enzyme activities, uptake of $\text{CO}_2/\text{HCO}_3^-$ and glycine, nuclear reproduction, exponential growth rate, and generation time. Some of these phenomena may involve light-stimulated metabolism of α -ketoglutarate via isocitrate to succinate and glyoxylate.

We report here that white light also affects the zoospore, the non-growing stage in this organism's life-cycle, by inducing encystment. This process involves a complex of very rapid, interrelated, structural and functional transformations (Cantino, Truesdell & Shaw, 1968; Soll, Bromberg & Sonneborn, 1969; Truesdell & Cantino, 1970, 1971), and should be useful for studying cellular differentiation apparently unaccompanied by protein synthesis (Lovett, 1968; Soll & Sonneborn, 1971).

METHODS

The wild-type *Blastocladiella emersonii* was a strain continuously subcultured from the original isolate (Cantino & Hyatt, 1953). Zoospores were produced by flooding single-generation OC (ordinary colourless) plants grown in darkness at 24° on Difco 'Cantino PYG Agar'. After 15 min. the spore suspension was decanted, passed through filter paper, and used immediately. Population densities were measured by fixing zoospores in 5% (v/v) glutaraldehyde and counting in a Speirs-Levy Eosinophil chamber. Encystment was studied by putting spore suspensions in 50 ml. Erlenmeyer flasks shaken at 76 oscillations/min. at 22° in a Warburg bath (Precision Scientific), and either illuminated from below with a built-in incandescent light bank, (3200 $\mu\text{W}/\text{cm}^2$ at bottom of flasks) or kept dark with

black paint or aluminium foil. Samples were removed at intervals, fixed as above, and scored for percent encystment. The effect of light intensity upon encystment was studied with 50 ml. flasks coated with various thicknesses of silver by the Rochelle Salts method (Hodgman, 1952-3). Their transmission, ranging from about 3 % to 100 %, was determined with an incandescent lamp and a Weston electric light meter. The transmission of colour filters (Edmund Scientific) was determined with a Beckman DU spectrophotometer and Gilford attachments.

Fig. 1. Effect of light ($3200 \mu\text{W}/\text{cm}^2$) on encystment of zoospores. (a) Suspensions (8.5×10^5 spores/ml.) exposed continuously to light, L, and darkness, D. (b) Suspensions (5.4×10^5 spores/ml.) maintained in continuous darkness, D, or transferred from darkness to light after 90, 270, and 420 min. respectively.

RESULTS AND DISCUSSION

When freshly harvested and filtered zoospores from dark-grown plants were incubated in the dark, encystment of the population commenced but quickly levelled off (Fig. 1a). When a sample of the same suspension was illuminated, further encystment occurred, but only after a lag.

If spores were incubated in the dark for 90 min. before they were illuminated, encystment was stimulated by light (Fig. 1b), but the initial lag period was shortened. When the spore population being incubated in the dark was left (e.g. for 270 or 420 min.) to reach its encystment plateau before it was illuminated, the lag period preceding light-induced encystment was further reduced or entirely eliminated. In a previous study (Truesdell & Cantino, 1971) with all three encystment inducers used (low temperatures, sulphonic acid dyes, and KCl) the mean time of encystment for a spore population was always very much lower than it is for light-induced encystment. As shown above, the length of the dark period preceding illumination of a spore population was inversely related to the length of the lag period at the beginning of encystment and therefore to the mean time of encystment. These results are consistent with the possibility that a swimming spore derived from dark-

grown plants must be altered in some way before it can encyst in response to light; preliminary evidence not reported here suggests that its DNA-containing γ -particles (Myers & Cantino, 1971) may be involved.

Fig. 2. Effect on encystment of transferring zoospores to darkness after exposure to light ($3200 \mu\text{W}/\text{cm}^2$). Suspensions (1.2×10^6 spores/ml.) either left in light, L, or transferred to darkness at 60 or 240 min. Arrows indicate time of transfer.

Table 1. *Encystment maxima in darkness and white light ($3200 \mu\text{W}/\text{cm}^2$) at various population densities*

Population density (spores/ml.)	Maximum encystment (%)	
	Darkness	Light
2.8×10^6	28	50
2.1×10^6	< 5	51
1.9×10^6	19	52
1.7×10^6	13	42
1.6×10^6	6	32
1.3×10^6	23	73
1.2×10^6	9	62
1.1×10^6	8	65
9.2×10^5	10	84
8.5×10^5	18	82
8.1×10^5	20	70
7.2×10^5	< 5	93
5.3×10^5	10	88
5.0×10^5	5	86
3.5×10^5	> 90	> 90
2.8×10^5	> 75	> 90

With suspensions of above 10^6 spores/ml. the maximum encystment induced by light seldom reached 70 % (Table 1). In the range 5×10^5 to 10^6 spores/ml., encystment in light was usually over 80 %, while in the dark it was generally under 20 %. At population densities

below 5×10^5 spores/ml., encystment in the dark approached the high level obtained in light, hence a light effect was not observed. Population density was therefore a critical factor for encystment in the dark; if this relationship was a consequence of 'self inhibition' (Truesdell & Cantino, 1971), visible light may have been functioning, in part at least, by overcoming its influence. It may be significant that in the media used by Soll & Sonneborn (1969), not tested in the present study, germination synchrony was independent of spore concentration up to 4.2×10^5 cells/ml., whereas above 5.9×10^5 cells/ml., population density exerted inhibitory effects.

Fig. 3. Effect of growing parental OC (ordinary colourless) plants in light ($250 \mu\text{W}/\text{cm}^2$, 24°) on the encystment capacity of the zoospore suspension (5.0×10^5 spores/ml.) produced by them. The suspension was either maintained in darkness, D, or transferred to light after 90 min., L.

Light-induced encystment ceased abruptly when spores were transferred to darkness (Fig. 2). Attempts were made with filters to ascertain which regions of the visible spectrum were the most effective for inducing encystment. Preliminary results suggested that blue light (400 to 500 nm.) was effective, but other wavelengths also seemed to play a role.

Concentrations of cycloheximide which inhibit growth of OC plants (Cantino & Hyatt, 1953), young germlings (Matsumae & Cantino, 1971) and spore germination (Lovett, 1968; Soll & Sonneborn, 1971) did not inhibit encystment (Lovett, 1968; Soll & Sonneborn, 1971). Whether cycloheximide could influence light induction of encystment was examined. A spore suspension (6.75×10^5 spores/ml.) was incubated in the dark at 22° for 90 min. with $0.25 \mu\text{g}$. cycloheximide/ml.; another suspension without cycloheximide was set up

simultaneously. Half the suspension containing cycloheximide was then exposed to 3200 $\mu\text{W}/\text{cm}^2$ of light and half was kept dark. The suspension without cycloheximide was then tested for the effect of cycloheximide (at two concentrations: 0.25 $\mu\text{g.}/\text{ml.}$ and 25.0 $\mu\text{g.}/\text{ml.}$) upon light-induced encystment. In no instance was light-induced encystment affected by the presence of cycloheximide.

When OC plants were grown under 250 $\mu\text{W}/\text{cm}^2$ of 'cool white' fluorescent light instead of darkness, and the zoospores derived therefrom tested as above, the difference in response of spores to light and darkness disappeared (Fig. 3). There is already ample evidence that the prior history of parental plants from which zoospores are derived may affect the γ -particles in a spore and alter the cell's composition or eventual behaviour (Cantino & Hyatt, 1953; Cantino & Horenstein, 1956*b*; Cantino, 1969; Matsumae, Myers & Cantino, 1970). It is now shown that the lighting conditions imposed upon an OC plant can also influence the spores eventually derived from it, enabling them to encyst in total darkness.

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