Fluorescence in the Migrating Pseudoplasmodium of the Cellular Slime Mold

Dictyostelium mucoroides

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ABSTRACT. Fluorescence was observed under light microscope in living cells and cell mass of Dictyostelium mucoroides. The fluorescence was localized in the vacuoles of live vegetative cells. While the cell mass of D. discoideum does not have a stalk during migration period, the cell mass of D. mucoroides has a stalk that forms at the beginning of the migration period. We were able to observe a preferential loss of the fluorescent vacuoles from the cells of the stalk and from the stalk-forming cells at the tip region of the slug. Although the fluorescence was also present in the mature spore mass of D. mucoroides, the fluorescence was not observed in the spores, but rather in the spaces between the spores within the spore mass. The fluorescent vacuoles in the cells of vegetative stage and of migrating slug stage may be related to the interspore fluorescence in the spore mass. Possible roles of the fluorescent substance(s) in amoebae, slugs and spore masses were discussed.

Amoebae of the cellular slime mold Dictyostelium mucoroides proliferate by binary fission, using bacteria as a food source. After deprivation of bacteria, the homogeneous population of slime mold cells aggregates to form a slug-shaped mass of cells (pseudoplasmodium) on a solid substratum. The cell mass of D. mucoroides has a stalk that forms at the beginning of the migration period, during which time the cell mass crawls over the surface of the substratum. Eventually the mass of cells generates a fruiting body, which consists of a mass of spores and a supporting cellular stalk.

The cells grown in liquid nutrient medium remain stable for an extended period of time prior to cell lysis (18). This period is referred to as the stationary phase. There have been several reports that stationary phase cells are significantly different from either growing or developing cells (14, 16, 23).

It is known that the cellular slime molds contain fluorescent substance(s). A major intracellular fluorescent product of D. discoideum cells, dictyopterin, 6-(D-threo-1,2-dihydroxypropyl)-pterin, has been isolated from vegetative cells (12). It has been reported that D. discoideum cells secrete lumazine (the demination product of pterin), an extracellular fluorescent product that has been linked with their ability to aggregate (19, 20). We previously observed fluorescence in the live cells of D. discoideum during their growth and morphogenesis (21). In present study, we examined the localization of fluorescence in the cells and cell mass of D. mucoroides by fluorescence microscopy. While the cell mass of D. discoideum forms a stalk after the migration period, the cell mass of D. mucoroides has a stalk from the start of the migration period.

MATERIALS AND METHODS

The cellular slime mold, Dictyostelium mucoroides, was used in all experiments. The cells were grown in a liquid medium that contained the following per liter: 10 g bacto-peptone (Difco, Detroit, U.S.A.), 10 g glucose, 0.96 g Na2HPO4·12H2O, and 1.45 g KH2PO4 (22). Escherichia coli strain B/r was the food source for the myxoamoebae. Cultures in 30 ml of the nutrient broth were shaken on a reciprocating shaker (100 strokes per min) at 22°C. The cells were harvested in the middle of the logarithmic phase of growth (2-5 × 10⁶ cells /ml), in the full growth phase (2-3 × 10⁷ cells/ml) and in the stationary phase, that is, the full grown cells were further incubated for 24 h in the same medium. The cells were washed free of bacteria with Bonner’s salt solution (2), and then observed by fluorescence microscopy.

To obtain cell masses cells were grown on solid nutrient medium with E. coli as the food source. Cells were harvested with a glass rod. The solid nutrient medium contained the same constituents as described above for the liquid medium except for the addition of 20 g of agar (Difco, Detroit, U.S.A.) per liter (2). Cells (2 × 10⁶) were washed free of bacteria with Bonner’s salt solution and allowed to develop on a square cel-lophane membrane (about 1 square cm) placed on 2% plain

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agar in a humid atmosphere at 22°C. After an appropriate incubation period, the square cellophane membrane, on which the slugs and fruiting bodies had developed, was transferred onto a glass slide, covered with a glass coverslip, and slightly compressed by the coverslip for microscopic examination. A slug was squeezed between the glass slide and the coverslip for observation of individual cells. For observation of individual spores, a spore mass was smeared onto a glass slide.

Fluorescence microscopy was carried out using a Nikon Microphot microscope (Nihonkougaku Co., Tokyo Japan) equipped with filter sets for selective autonomous/FITC (fluorescein isothiocyanate) fluorescence. The excitation wavelength was around 365 nm and the emission wavelength was cut off below 410 nm. Photographs were taken on Fuji color film with ASA 100.

RESULTS

Fluorescent vacuoles were observed in the growing amoebae of *Dictyostelium mucoroides* (Fig. 1). The intensity of the fluorescence of the vacuoles decreased in the full grown cells, compared with that of cells in the middle of the logarithmic growing phase. Further decrease of the intensity of fluorescence of the vacuoles occurred in the stationary phase cells which were incubated for the extended period of time (24 h) in the same medium after full growth.

The cell mass of *D. mucoroides* has a stalk that forms at the beginning of the migration period, in contrast to the cell mass of *D. discoideum*. The stalk cells are formed in the tip region of the slug. Figure 2 shows bright fluorescence over the whole slug, although the fluorescence is weak in the tip region of the slug as well as in the stalk which is located in the cylindrical axis of the migrating slug. To observe the fluorescent vacuoles of the cells, a slug was squeezed between a glass slide and a coverslip. Figure 3 shows the loss of fluorescent vacuoles from the cells in the tip region and those in the stalk. As shown in Fig. 4, fluorescent vacuoles were maintained in almost all cells located in the fluorescent region of a slug. This observation suggests that the fluorescent vacuoles are preferentially lost from the cells involved in the process of stalk cell differentiation. The fluorescence lost from stalk cells was not released into the external environment of the slug at a level that could be detected microscopically.

In the mature fruiting body, the spore mass was fluorescent, although the fluorescence was not present in the individual spores, but rather present in the spaces between the spores (Fig. 5). This is consistent with the results from studies of the spore mass of *D. discoideum* as described elsewhere (21).

DISCUSSION

In this study we observed the localization of fluorescence in amoebae, slugs, and spore masses of *D. mucoroides*. Our observations coincide with those previously made for *D. discoideum* (21), the exception being the loss of fluorescent vacuoles from the tip region of *D. mucoroides*. It has been reported that *D. discoideum* cells secreted fluorescent substance (lumazine), which is responsible for the extracellular fluorescent products linked with their aggregation ability (19, 20). In *D. mucoroides* cells, we observed the loss of the fluorescent vacuoles in the cells of the tip region of the slug and in the stalk cells. In fruiting body, the stalk cells and the spores themselves did not show fluorescence, but the

**Fig. 1.** Corresponding light and fluorescence micrographs of growing *D. mucoroides* cells. Light micrograph of cells (A) and fluorescence micrograph of cells (B). Bright fluorescent vacuoles are present in the cells. Scale bar indicates 50 μm.
surroundings of the spores did. The fluorescent vacuoles in the cells of vegetative stage and of migrating slug stage may be related to the interspore fluorescence in the spore mass.

As to the fluorescence in the stalk region of *D. discoideum* (21), we might observe false or residual fluorescence, since, as to be described elsewhere, fluorescence spectra using a microspectrophotometer suggested the existence of non-specific and low noisy fluorescence in the stalk region. The above observation coincides with the weak fluorescence in the stalk region of *D. mucoroides* observed in this paper.

In *Saccharomyces cerevisiae*, the fluorescence in the culture medium has been attributed to NAD(P)H (9). In *Escherichia coli*, an unknown fluorophore(s) that displayed an emission spectrum very similar to that of NAD(P)H was shown to be a biochemically distinct compound. This fluorophore(s) is responsible for the fluorescence of a culture of *E. coli*, with fluorescence being emitted by both the cells and the medium (9). The fluorescence of dictyopterin, a major pteridine isolated from vegetative cells of *D. discoideum* (12), is very similar to that of NAD(P)H in mitochondria (1). The contribution of natural fluorophores to the fluorescence observed is still unknown in *D. mucoroides* cells.

The fluorescent substance(s) is thought to be involved in the photoreception associated with phototaxis. Localized fluorescence has been widely observed in the flagella and/or the eyespot of brown and golden algae (3, 10, 11). By microspectrophotometry, pterin- and flavin-like fluorescent substances were observed in the paragellar body of *Euglena gracilis* (17). Furthermore, the pterin- and flavin-like fluorescent substances were extracted from isolated flagella of *Euglena gracilis* (7). Since *D. discoideum* amoebae also exhibit phototactic responses (4, 8), the fluorescent vacuoles in the cellular slime mold might be involved in the photoreception as-
associated with phototaxis.

Schmidt et al. (17) reported the presence of blue fluorescent spots that were distinct from the fluorescent paraflagellar body of Euglena gracilis, although the functional role of the former fluorescence was unclear. In Dictyostelium, the functional role of the fluorescent substance(s) in amoebae requires further investigation.

Although slugs demonstrate phototaxis (13), the phototactic response of the slug is based on a lens effect of the cylindrical body which consists of about $10^5$ cells (5, 6). Furthermore, as demonstrated in this study, fluorescent vacuoles are preferentially lost from the cells at the tip region of the slug. In the slug, therefore, it may be hard to correlate phototaxis with the functional role of the fluorescent substance(s).

Ultraviolet irradiation of washed spores of D. discoideum resulted in a prolonged delay of the emergence of amoebae from swollen spores (15). Since the fluorescent substance(s) absorbs harmful ultraviolet light and emits the fluorescent light in a harmless form, the fluorescent substance(s) may play a role in protecting the spores from ultraviolet light in the field. Furthermore, spores are capable of continuing to the next generation, while stalk cells are not. This fact is consistent with the observations presented in the present paper, namely, that the fluorescent substance(s) is present in the spaces between the spores and surrounds the spores in the spore mass (Fig. 5), but it is not present in either the stalk or the stalk-forming region (Fig. 3).

**Fig. 4.** Fluorescence micrograph of the bright fluorescent region in a slug that has been compressed between a coverslip and a glass slide. Almost all cells contain bright fluorescent vacuoles. Scale bar indicates 50 μm.

**Fig. 5.** Fluorescence micrograph of spores smeared on a glass slide. Note that the spores are not fluorescent, but that the interspore space is. Scale bar indicates 50 μm.

**REFERENCES**


Fluorescence in the Cell Mass of D. mucoroides


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