

Possible Existence of a Light-Inducible Protein That Inhibits Sexual Cell Fusion in *Dictyostelium discoideum*

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ABSTRACT. Sexual cell fusion is an initial step of macrocyst formation in *Dictyostelium discoideum* and requires environmental conditions such as darkness, plenty of water and the presence of calcium ions. We have been analyzing the mechanism of sexual cell fusion between HM1 and NC4, heterothallic strains in *D. discoideum*. Cells of these strains have been shown to be fusion competent when cultured in a liquid medium in darkness, but not so when cultured on agar plates or in a liquid medium in the light. Two cell-surface proteins, gp70 and gp138, have been identified as target molecules for fusion-blocking antibodies and therefore as relevant to sexual cell fusion.

In the present study, gp70 was shown to be present in HM1 cells cultured in the light, and fusion incompetent. Intact HM1 cells cultured in the light were unable to absorb the fusion-blocking activity of antibodies against membrane components of fusion-competent HM1 cells, whose activity had been shown to be absorbed by gp70, but they did so after separation of proteins in the SDS-PAGE. In addition, fusion-competent HM1 cells were found to lose their fusion competence by subsequent cultivation in the light. This loss of competence was cycloheximide sensitive, indicating that *de novo* synthesis of proteins was necessary for this inhibition. From these results, we presume that light induces a protein that hinders the interaction of gp70 in HM1 cells with its receptor on the NC4 cell surface and thereby inhibits the sexual process between these strains.

The soil amoebae cellular slime molds usually propagate as single haploid cells and, upon starvation, gather and form a multicellular structure to asexually develop into fruiting bodies. They also sexually develop into macrocysts under certain environmental conditions (1, 7). As the first step of this sexual development, the amoeboid cells fuse to form zygotic cells called giant cells, which subsequently gather and engulf surrounding cells to develop into macrocysts (2, 9). Conditions such as darkness, a liberal supply of water and the presence of calcium ions are preferable for macrocyst formation (2, 4, 8). Thus, the molecular basis for cellular response to the environmental factors is one of the topics on the sexuality of the cellular slime mold.

We have been studying the mechanism of sexual cell fusion using HM1 and NC4, the opposite mating-type strains in *D. discoideum* (3). Establishment of an experimental system for synchronous and extensive cell fusion enabled us to conduct detailed analysis of the sexual cell fusion (10). Most of the environmental factors described above were shown to affect the step of cellular acquisition of competence for sexual cell fusion, except for calcium ions which were required for the actual process of cell fusion (11). Growth-phase cells of HM1

and NC4 on agar plates in the light are incapable of undergoing sexual cell fusion (fusion incompetent). When such cells are cultured in a liquid medium in darkness, they acquire the ability to fuse and become fusion-competent cells. Light inhibits this process and HM1 cells cultured in a liquid medium in the light are unable to fuse with NC4 cells. NC4 cells are less sensitive to light than HM1 cells and have fusion competence to some extent even when cultured in the light (10).

Two membrane proteins, gp70 (5, 13) and gp138 (12), have so far been identified as relevant to sexual cell fusion between HM1 and NC4. The former is present in HM1 cells but not in NC4 cells, while the latter is present both in HM1 and NC4 cells. Therefore, we suspect that gp70 is responsible for cell recognition and gp138, for membrane fusion. In the present study, we intended to analyze the manner by which light affects the expression and/or function of these fusion-related molecules.

The conditions for cell culture and fusion assay were the same as in our previous reports (5, 10–13). We first examined whether or not gp70 was present in HM1 cells cultured in a liquid medium in the light (light-cultured HM1 cells). As we showed in our previous paper, Fab fragments of rabbit antibodies raised against membrane components of HM1 cells (Fab) blocked cell fusion be-

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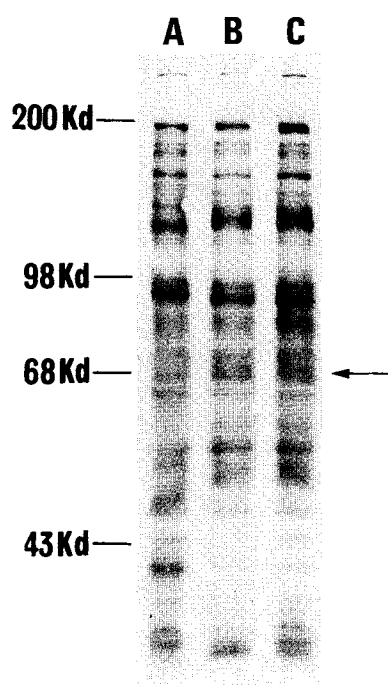


Fig. 1. Immunoblotting patterns showing the presence of gp70 in light-cultured HM1 cells. A: fusion-incompetent HM1, B: fusion-competent HM1 and C: light-cultured HM1 cells. The same number of cells (1×10^5 cells) was loaded on each lane. The arrow indicates the position of gp70.

tween HM1 and NC4 (13). This fusion-blocking activity of Fab was absorbed by fusion-competent HM1 cells, and gp70 was shown to be responsible for this absorption.

In an immunoblotting pattern of light-cultured HM1 cells, an antigenic protein was detected at the apparent position of gp70 (Fig. 1). However, when the Fab was incubated with intact light-cultured HM1 cells, it scarcely lost the fusion-blocking activity (Fig. 2). The extent of inhibition was completely the same as that of Fab absorbed with fusion-incompetent HM1 cells cultured on the agar plates in the light which did not possess gp70. In an attempt to determine whether gp70 detected in light-cultured HM1 cells possesses the activity to absorb fusion-blocking Fab, membrane components of light-cultured cells were electrophoretically separated in a SDS-polyacrylamide gel (6) and used for absorption after blotting to nitrocellulose membrane (13). They absorbed the Fab far more than did fusion-incompetent HM1 cells, whose ability was at the background level, and to a degree comparable to that of fusion-competent HM1 cells (Table 1). These results indicate that light-cultured HM1 cells do retain a target molecule for the fusion-blocking antibody, gp70, but it exists in intact cells in a state such that binding of antibodies is not feasible. In such a situation, molecules on the surface of

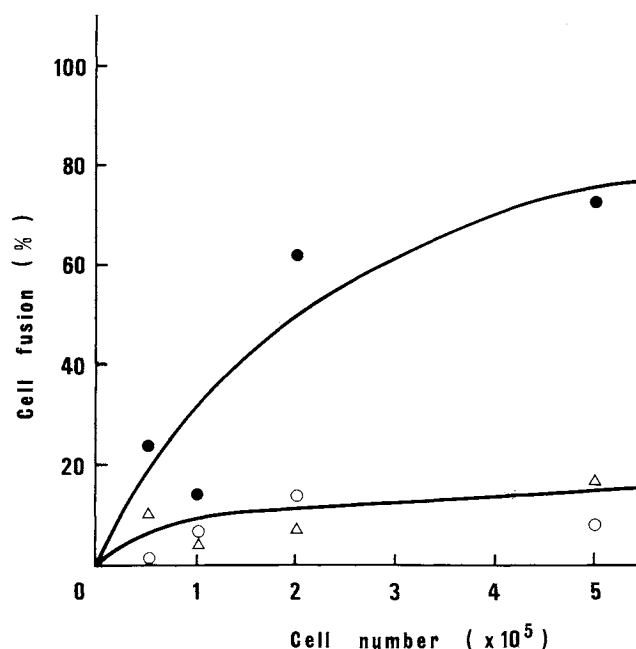


Fig. 2. Restoration of cell fusion in the presence of Fab absorbed with intact HM1 cells. Fusion-competent (●), light-cultured (△), fusion-incompetent (○) HM1 cells were incubated with 0.5 mg of Fab on ice for 1 hr with occasional mixing. At the end of incubation, the cell-Fab mixture was centrifuged, and the supernatant was assayed for fusion-blocking activity. Assays were performed in duplicate and the means were plotted.

NC4 cells would also be incapable of interacting with gp70, resulting in fusion incompetence of light-cultured HM1 cells.

In relation to this, fusion-competent HM1 cells were found to lose their fusion competence by subsequent cultivation in the light. Prolonged cultivation in darkness did not diminish fusion competence, if the culture was properly diluted to avoid starvation (11). Interestingly, if cycloheximide was added to the medium at 100 $\mu\text{g}/\text{ml}$ during this light cultivation, reduction of fusion

Table 1. BLOT ABSORPTION OF FUSION-BLOCKING Fab

Cells	Absorption (%)
Fusion-competent HM1	91.6 ± 1.6
Light-cultured HM1	73.5 ± 9.4
Fusion-incompetent HM1	30.0 ± 2.5
None ^a	33.5 ± 8.5

Crude membrane fraction was obtained by freeze-thawing 6×10^7 HM1 cells and was separated in a preparative 7.5% polyacrylamide gel. Proteins in the gel were transferred to nitrocellulose membranes and the area containing gp70 (60–80 kDa) was excised and incubated with 0.5 mg of Fab. After the incubation, the absorbed Fab was assayed for fusion-blocking activity. Assays were performed in duplicate, and the mean \pm SD values are shown.

^aA control filter without protein blot.

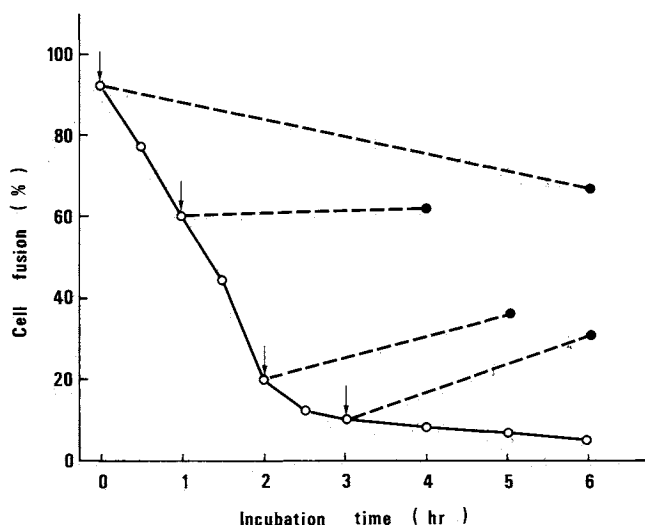


Fig. 3. Effect of cycloheximide on the light-induced reduction of fusion competence in HM1 cells. Fusion-competent HM1 cells (3×10^6 /ml) were cultured in the light and assayed for fusion competency at 30-min intervals of cultivation (○—○). At the times indicated by arrows, cycloheximide was added to 100 μ g/ml, and cells were cultured for an additional 3–6 h and then assayed for fusion competency (●—●). Assays were performed in duplicate and the means were plotted.

competence discontinued (Fig. 3), indicating that *de novo* synthesis of proteins is necessary for light-induced loss of fusion competence. This suggests that HM1 cells cultured in the light are unable to fuse not because they lack molecules necessary for cell fusion, but because they carry an inhibitory protein.

How the light-inducible inhibitory protein prevents the function of gp70 is unknown. We may assume that the protein could directly interact with gp70 to mask it, or it may indirectly affect the localization of gp70. Efforts to detect light-induced iodinated protein on the HM1 cell surface have so far been unsuccessful, although this does not necessarily exclude the former possibility. In any case, it is evident that there is a third class of protein in relation to sexual cell fusion between HM1 and NC4. Thus, the mechanism of sexual cell fusion appears to be achieved by the coordinated interactions of multiple molecules, which are finely regulated by environmental factors.

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