Distribution of gp138, a cell surface protein responsible for sexual cell fusion, among cellular slime moulds

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(Received 23 June 1992; revised 21 September 1992; accepted 5 October 1992)

Sexual cell fusion occurs between NC4 and HM1, heterothallic strains of Dictyostelium discoideum. Several cell surface proteins relevant to the process have been identified. One of them, gp138, exists in fusion-competent cells of both NC4 and HM1, and is considered to be more concerned with membrane fusion than gamete recognition. In this study, we raised monoclonal antibodies against gp138 and examined gp138 distribution among strains and species of cellular slime moulds to confirm its importance in sexual cell fusion. All heterothallic and bisexual D. discoideum strains examined were found to possess gp138, while asexual and homothallic strains lacked it. The anti-gp138 monoclonal antibody detected several distinct proteins in homothallic strains and one in an asexual strain. Some of the former proteins appeared together with the increase in binucleated cells. Cells of Dictyostelium mucoroides and Polysphondylium pallidum did not possess proteins reactive to the monoclonal antibody. These results indicate that gp138 is common among, but restricted to, cross-matable strains of D. discoideum. Our results also support previously published molecular phylogenetic studies which suggest that homothallic and asexual strains of D. discoideum are remote from other strains of D. discoideum but are less distantly related to them than other species are.

Introduction

Sexual reproduction provides a means for shuffling genetic material. Without it, accumulation of advantageous mutations would be much slower (Kirkpatrick & Jenkins, 1989) and deleterious ones could not easily be eliminated (Kondrashov, 1988). Analysis of the origin and evolution of sexuality, therefore, is an interesting and important subject of basic biology, but its molecular basis is not well documented. Although molecular mechanisms of sexual cell fusion are being elucidated in several lower (Luporini & Heckmann, 1990; Herskowitz, 1989; Glabe et al., 1991) and higher organisms (Sidhu & Guraya, 1991), very little phylogenetic analysis has been carried out.

The soil amoebae of the cellular slime moulds are unique and useful organisms for the study of sexual phenomena. They feed on bacteria and propagate by fission, while in due course they enter into the sexual cycle called macrocyst formation (Blaskovics & Raper, 1957; Maclnnnes & Francis, 1974). Entrance into the sexual cycle is controlled by environmental factors such as light and excess water (Nickerson & Raper, 1973; Erdos et al., 1976). The mating system of cellular slime moulds is polymorphic. Heterothallic, homothallic, bisexual and asexual strains have all been found in Dictyostelium discoideum (Erdos et al., 1973; Robson & Williams, 1980) and alternate homothallism (Blaskovics & Raper, 1957) or heterothallism (Clark et al., 1973; Erdos et al., 1975; Francis, 1975) have been described in other species.

We have been studying the molecular aspects of sexual cell fusion in macrocyst formation using NC4 and HM1, complementary heterothallic strains in D. discoideum. Several cell surface components with possible relevance to the cell fusion between these strains have been identified (for a review, see Urushihara, 1992). One of these, gp138, has been shown to be a target antigen for cell-fusion-inhibiting antibodies (Suzuki & Yanagisawa, 1989b) and has been purified and characterized (Suzuki & Yanagisawa, 1990). This protein is detected in fusion-competent cells of both HM1 and NC4 and is therefore suspected to be responsible for membrane fusion and not for gamete recognition.

In this study, we raised monoclonal antibodies against gp138 and examined whether D. discoideum strains other than NC4 and HM1, and species other than D. discoideum, possessed gp138. It was widespread among heterothallic and bisexual strains in D. discoideum. Homothallic and asexual strains of D. discoideum lacked...
gp138 but retained proteins which shared the epitope to the antibody with gp138; other species did not.

Methods

Organisms and culture methods. NC4 (mat A) and HM1 (mat a, derived from V12) are heterothallic strains of D. discoideum and their mating properties have been described in detail (Suzuki & Yanagisawa, 1989a). WS472, WS583 and WS655 are mat A and WS7 and WS656 are mat a strains of D. discoideum (Erdos et al., 1973; Robson & Williams, 1979), and were kindly provided by Dr K. Williams (Macquarie Univ., NSW, Australia). WS2162 and WS112B, bisexual strains which form macrocysts with either mat A or mat a strains (Robson & Williams, 1980), and WS584, an asexual strain without sexual potency (Erdos et al., 1973), were gifts from Dr Welker's laboratory (Utah State Univ., USA). AC4 (Cavender & Raper, 1968) and ZA3A (Robson & Williams, 1980) are homothallic strains. These asexual and homothallic strains have been classified as being D. discoideum from morphological criteria, their fruiting bodies having discs at the bottom of the stalks, but recently this classification has been questioned (Briscoe et al., 1987; Evans et al., 1988) (see Discussion for details).

The Dm7 strain of Dicystostelium mucoroides (MacInnes & Francis, 1974) is homothallic and was provided by Dr Amagai (Tohoku Univ., Aoba, Sendai, Japan). A heterothallic strain of Polysphondylium pallidum, CK5 (Mizutani et al., 1990), was isolated by Hagiwara. The names and mating properties of the strains used, which were confirmed at the beginning of the study, are given in Table 1. Each strain was maintained as fruiting bodies on nutrient SM-agar plates (Sussman, 1966) with Klebsiella aerogenes as a food source.

Preparation of fusion-competent cells. To obtain cells competent for sexual cell fusion (fusion-competent cells), growth-phase cells on SM-agar plates were cultured at 22 °C in a shaker liquid medium in darkness for 15 h unless otherwise stated. This culture has been shown to render NC4 and HM1 cells capable of immediate cell fusion (Saga et al., 1983).

Partial purification of gp138. gp138 was partially purified using WGA affinity columns (Suzuki & Yanagisawa, 1990). Cell ghosts (crude membrane fraction) (Sussman & Boschwitz, 1975) were prepared from fusion-competent NC4 cells and solubilized in a Tris buffer (10 mm-Tris/HCl, pH 7.4) containing 0.5% cholic acid and 0.5% deoxycholic acid and centrifuged at 14000 r.p.m. for 10 min. The supernatant was recentrifuged for clarification and loaded onto a column of WGA-agarose equilibrated with the Tris buffer containing 0.1% deoxycholic acid (DOC-Tris). After extensive washing with DOC-Tris, the bound fraction was eluted with DOC-Tris containing 0.2 M-N-acetyl-D-glucosamine.

Raising and screening hybridomas. Partially purified gp138 was intraperitoneally injected into BALB/c mice together with aluminium hydroxide as adjuvant. After three weeks, the same quantity of gp138 was injected into their eye sockets. Three days later, the mice splenocytes were fused with P3U1 (P3X63Ag8-U1) myeloma cells by the conventional hybridoma technique. The resulting hybridomas were first screened for production of antibodies that bound to partially purified gp138 by the enzyme-linked immunosorbent assay. The selected hybridomas were further screened for specificity to gp138 by immunoblotting. Positive hybridomas were then cloned by plating at the limiting dilution.

Gel electrophoresis and immunoblotting. Slab SDS-PAGE was performed by the method of Laemmli (1970) with minor modifications. For immunoblotting, proteins in a gel were transferred to a nitrocellulose membrane (0.45 µm, Schleicher & Schuell), which was then incubated with antibodies for 1 h at room temperature. The membrane was further incubated with peroxidase-conjugated anti-mouse IgG (Jackson) or protein A (EY Laboratories) for another hour and then developed with 0.03% dianaminobenzidine.

Nuclear staining. To estimate the extent of cell fusion in the culture of homothallic strains, aliquots of cell suspension were taken for nuclear staining with Hoechst 33258 by the method described previously (Urushihara et al., 1990). Numbers of cells with 1, 2, 3 and more nuclei were counted and the percentage of nuclei in multinucleated cells to the total number of nuclei was calculated and used as the index of cell fusion. Duplicates of more than 200 cells were examined for each sample.

Results

Specificity of the anti-gp138 monoclonal antibody

Five monoclonal antibodies specific to gp138 were obtained. One of them, EF11, was used for this study. It gave a single band in immunoblots of both total proteins of fusion-competent NC4 cells and of partially purified gp138 (Fig. 1). This band comigrated with gp138 detected by FRA-6, a rabbit antiserum raised against purified gp138 (Suzuki & Yanagisawa, 1990). Specificity of EF11 to gp138 was confirmed also by two-dimensional gel electrophoresis by the methods of Hirabayashi (1981) (data not shown).

Detection of gp138 in heterothallic strains of D. discoideum

Various heterothallic strains of D. discoideum were examined for expression of gp138 after cultivation in a liquid medium for 15 h in darkness (Fig. 2). EF11
detected gp138 in HM1. This is in accord with the previous finding that, although gp138 was first identified in NC4, it exists also in HM1 (Suzuki & Yanagisawa, 1989b). Furthermore, all other heterothallic strains in *D. discoideum* examined also possessed gp138. These included both *mat A* (WS472, WS583 and WS655) and *mat a* strains (WS7 and WS656). An additional band of about 95 kDa is seen in Fig. 2 but it appeared without reproducibility. This band seems to be due to background staining. Except for this, gp138 was the only protein that reacted with EF11 in all the heterothallic strains shown in Fig. 2.

**Reactivity of anti-gp138 antibody with other strains of *D. discoideum***

We extended the examination to nonheterothallic strains, that is, bisexual, homothallic and asexual strains which had been morphologically assigned to *D. discoideum*. As can be seen in Fig. 3, a bisexual strain, WS2162, possessed gp138 as the only band detected by EF11.

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**Fig. 2.** Detection of gp138 in heterothallic strains of *D. discoideum*. Whole proteins obtained from $1 \times 10^4$ fusion-competent cells of (a) NC4, (b) WS472, (c) WS583, (d) WS655, (e) HM1, (f) WS7, and (g) WS656 were subjected to SDS-PAGE, transferred to nitrocellulose membrane and immunostained with EF11. Lanes a–d are *mat A*, and e–g *mat a* strains. The arrow indicates the position of gp138.

**Fig. 3.** Detection of gp138 in atypical strains of *D. discoideum* and in other species. Cells were cultured in a shaken liquid medium to render them fusion-competent as described in Methods. Whole proteins obtained from $1 \times 10^4$ cells of (a) NC4, (b) WS2162, (c) WS112B, (d) AC4, (e) ZA3A, (f) WS584, (g) Dm7 and (h) CK8 were subjected to SDS-PAGE and either transferred to nitrocellulose membrane for immunostaining with EF11 (A) or directly stained with CBBR (B). The arrow indicates the position of gp138.
Fig. 4. Time course of multinucleated-cell formation (a) and appearance of EF11-reactive proteins (b) in a liquid culture of AC4 cells. Cells on SM-agar plates were cultured in a liquid medium at 22 °C in darkness. At the times indicated, cells were taken for nuclear staining and for SDS-PAGE followed by immunostaining with EF11. In (a) duplicate samples were examined and their means were plotted. Standard deviations are shown by bars. The arrow in (b) indicates a band appearing in parallel with formation of multinucleated cells.

Another bisexual strain, WS112B, gave similar results except that the band of gp138 tended to be somewhat broader. This tendency was also observed in HM1 (for example, lane e in Fig. 2) but not in NC4 or WS2162.

In contrast to the strains mentioned above, homothallic and asexual strains gave entirely different results. EF11 appeared to react with proteins of distinct molecular mass from gp138 in the immunoblot of homothallic strains, AC4 and ZA3A, and an asexual strain, WS584. AC4 possessed two EF11-reactive proteins of 190 and 96 kDa. ZA3A possessed a 66 kDa protein with enormous reactivity to EF11 and several proteins of 195, 57, 52, and 46 kDa with much weaker reactivity, while WS584 possessed a single EF11-reactive protein of 185 kDa.

Those EF11-reactive bands in AC4 and ZA3A may be related to sexual cell fusion. Alternatively, they might simply share the epitope to EF11 with gp138, having nothing to do with cell fusion. In order to clarify this point, occurrence of cell fusion and expression of EF11-reactive proteins in these strains were examined in parallel. To do this, growth-phase cells on SM-agar plates were cultured in a liquid medium in darkness, and at appropriate times, a small number of cells were taken from the culture and processed for nuclear staining to detect multinucleated cells and for electrophoresis to detect EF11-reactive proteins.

As shown in Fig. 4(a) the multinucleated cells in AC4 were barely above 1% at time 0; these probably represent cells in mitosis. The percentage of multinucleated cells gradually increased with time and reached a plateau at around 16 h. In the immunoblotting pattern of AC4 (Fig. 4b), the 96 kDa band was absent at time 0,
Table 1. Detection of proteins reactive to the anti-gp138 monoclonal antibody

<table>
<thead>
<tr>
<th>Species*</th>
<th>Strain</th>
<th>Mating property</th>
<th>gp138</th>
<th>Other†</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. discoideum</td>
<td>NC4</td>
<td>Heterothallic (mat A)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>WS472</td>
<td>Heterothallic (mat A)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>WS583</td>
<td>Heterothallic (mat A)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>WS655</td>
<td>Heterothallic (mat A)</td>
<td>-</td>
<td>+</td>
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<td></td>
<td>HM1</td>
<td>Heterothallic (mat a)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>WS7</td>
<td>Heterothallic (mat a)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>WS656</td>
<td>Heterothallic (mat a)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>WS2162</td>
<td>Bisexual</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>WS112B</td>
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<td>+</td>
<td>-</td>
</tr>
<tr>
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<td>+</td>
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<tr>
<td></td>
<td>WS84</td>
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<td>+</td>
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<td>D. mucoroides</td>
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<td>P. pallidum</td>
<td>CK8</td>
<td>Heterothallic</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Assignment of homothallic and asexual strains to D. discoideum does not seem to be appropriate (see text) but morphological classification is adopted in this instance.
† Molar mass of the reactive proteins were, 190 and 96 kDa in AC4, 195, 66, 57, 52 and 46 kDa in ZA3A, and 185 kDa in WS84.

Discussion

The cell surface glycoprotein gp138 was previously shown to be responsible for sexual cell fusion between NC4 and HM1, complementary heterothallic strains in D. discoideum (Suzuki & Yanagisawa, 1989b). The purpose of the present study was primarily to investigate the universality of this protein in order to confirm its importance, and secondly, to determine its relationship to the divergency of mating systems in cellular slime moulds. Our results demonstrated the presence of gp138 as a single protein reactive to EF11, an anti-gp138 monoclonal antibody, in all heterothallic and bisexual (typical) strains and homothallic and asexual (atypical) strains of D. discoideum and between D. discoideum and the other two species.

Absence of EF11-reactive proteins in species other than D. discoideum

Strains of different species from D. discoideum, Dm7 in Dictyostelium mucoroides and CK8 in Polysphondylium pallidum, were examined. Fusion-competent cells of Dm7 and CK8 were obtained by culturing cells in liquid for 18 h and 17 h, respectively, and were subjected to SDS-PAGE. When the electrophoresis gel was stained with Coomassie Blue, comparable amounts of proteins to D. discoideum strains were seen in both of the species (Fig. 3b). However, EF11 did not detect any proteins in these strains (Fig. 3a). Alternative methods to obtain fusion-competent Dm7 cells by moderate starvation (Filosa & Dengler, 1972) or cold treatment (Suzuki et al., 1992) were attempted. In neither case were EF-11 reactive proteins detected. Thus, it was concluded that D. mucoroides and P. pallidum did not possess gp138, nor proteins sharing the epitope to EF11 with gp138. All the above results of immunostaining are summarized in Table 1. Obvious discreteness is seen between heterothallic and bisexual (typical) strains and homothallic and asexual (atypical) strains of D. discoideum and between D. discoideum and the other two species.
and bisexual strains of *D. discoideum*, and seemed to be remote from typical (heterothallic and bisexual) *D. discoideum* strains. In our study, the former atypical *D. discoideum* strains did not possess gp138 and the latter typical ones possessed gp138. Secondly, however, their results indicated that the homothallic and asexual strains were less different from typical *D. discoideum* strains than were morphologically distinct species such as *D. mucoroides* and *P. pallidum*. The present results showed that the former possessed EF11-reactive proteins, while the latter did not possess any EF11-reactive proteins. In this respect, it may be worth noting that we previously reported that the cell-fusion promoting factor was shared in heterothallic and homothallic systems and suggested that their molecular mechanisms for sexual cell fusion might be similar (Urushihara *et al*., 1990). Finally, they claimed that, since two homothallic strains, AC4 and ZA3A, were different in protein and nucleic acid polymorphisms and not closely related, they should be assigned to separate species. We further demonstrated that patterns of antigenic proteins reactive to EF11, including the molecular entities correlated with formation of multinucleated cells, were different between AC4 and ZA3A.

One report suggests the possibility that homothallic strains undergo sexual cell fusion with heterothallic ones in *D. discoideum*. Robson & Williams (1980) obtained macrocysts in the co-culture of homothallic and heterothallic strains. Although their results indicate that homothallic strains can form macrocysts in the presence of heterothallic ones, this does not necessarily mean that sexual cell fusion occurred between those strains. It is also uncertain whether two homothallic strains mutually fuse to produce macrocysts or not. To clarify these points, genetic analyses should be carried out. Because of poor germination of macrocysts, this kind of study has not yet been done, but should ultimately be carried out.

The fact that homothallic and asexual strains did not possess gp138 and that *D. mucoroides* and *P. pallidum* did not possess any EF11-reactive proteins indicates dissimilarity in mechanisms of sexual cell fusion among those strains. However, it is premature at this stage of research to judge whether or not this is the reason for the absence of cross-mating with typical *D. discoideum* strains. Conversely, the polymorphism of EF11-reactive proteins might itself be the result of the lack of cross-mating, as simple accumulation of spontaneously-occurring independent changes would be enough to create protein polymorphisms among species without genetic exchange. Further analysis of molecular mechanisms of sexual cell fusion will elucidate these points.

This work was supported by research grants (Nos 01654502 and 01640502) from the Ministry of Education, Science and Culture of Japan to H. Urushihara. Maintenance and handling of animals were carefully carried out at the Laboratory Animal Research Center of the University of Tsukuba.

**References**


Distribution of a cell fusion protein


