SHORT COMMUNICATION

Quantitative Analysis of Macrocyst Formation in
*Dictyostelium discoideum*

By GILLIAN E. ROBSON AND KEITH L. WILLIAMS*†

*Genetics Department, Research School of Biological Sciences, Australian National University, Canberra, A.C.T., Australia*

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Quantitative studies are reported on macrocyst formation, the sexual cycle in *Dictyostelium discoideum*, under conditions in which asexual fruiting body formation is prevented. Light inhibited amoebae of both mating types from participating in macrocyst formation, and amoebae of the *mata* mating type responded better to a mating stimulus than amoebae of the *matA* mating type. These results are interpreted in terms of a possible two-way induction system leading to macrocyst formation in *D. discoideum*.

INTRODUCTION

The sexual cycle in the cellular slime mould *Dictyostelium discoideum* involves a one-locus, two-allele system (Erdos et al., 1973; Wallace & Raper, 1979; Robson & Williams, 1980) and its associated vegetative incompatibility (Robson & Williams, 1979). To complement recent genetic studies, it is important to understand the physiology and biochemistry of the sexual cycle (macrocyst formation) in *D. discoideum*. The general environmental requirements for macrocyst formation (moisture, Ca$^{2+}$, low phosphate concentration, darkness) are known (Blaskovics & Raper, 1957; Nickerson & Raper, 1973; Erdos et al., 1976; Filosa, 1979; O'Day & Lewis, 1981). However, the nature of the interactions between strains of opposite mating type, and in particular the role of mating hormone(s), is controversial. There is evidence for a one-way secretor–responder system involving a gaseous hormone (O'Day & Lewis, 1981) although some groups remain sceptical about the existence of mating hormone (Wallace, 1977; D. Bozzone, personal communication). A recent report on *Dictyostelium giganteum* suggests that the mating system in this species may involve two-way hormonal interactions (Lewis & O'Day, 1979).

Some of the controversy about the mating system in *D. discoideum* can probably be attributed to the assay used for macrocyst formation. In no case has a quantitative assay been used, and in several studies strains of opposite mating type were grown together and macrocyst production was tested under conditions also allowing asexual fruiting body formation. Under these conditions the test measures the *preference* for macrocyst (sexual) development versus asexual fruiting body formation, rather than whether or not macrocysts can be formed (see Filosa & Chan, 1972). In the work described here we have used the liquid culture technique of Filosa & Dengler (1972) to overcome problems of competition between sexual and asexual developmental pathways and to obtain quantitative results. We show that light prevents amoebae of both mating types from participating in macrocyst formation, and that amoebae of different mating type differ in their response to a mating stimulus. This represents evidence for a possible two-way induction in the sexual cycle of *D. discoideum*.

† Present address: Max-Planck-Institut für Biochemie, D-8033 Martinsried bei München, Federal Republic of Germany.
METHODS

Approximately 10^5 amoebae of strains V12 (mata2) and TS12 (matA1) (Robson & Williams, 1979) were plated separately on SM agar (Sussman, 1966) or 0.1% LP agar (Nickerson & Raper, 1973) together with Klebsiella aerogenes and allowed to grow for approximately 48 h in the dark (foil wrapped) or the light (Petri plates placed upside down beneath a daylight fluorescent tube) at 21 ± 1 °C. When most of the bacteria had been consumed, but before the amoebae started to aggregate, amoebae were harvested in cold sterile distilled water and washed three times to remove residual bacteria. Amoebae of each strain were finally resuspended in water at 5 x 10^7 ml⁻¹ and dispensed into Linbro tissue culture dishes (FB-16-24-TC) which contained 20 mM-CaCl₂ (final concentration). A suspension of 5 x 10^6 amoebae of one strain and an appropriate number of amoebae of the other strain was added to make a final volume of 1 ml per well. Viable counts of the washed amoebae were obtained by incubating low numbers with K. aerogenes on SM agar at 21 ± 1 °C and counting the number of colonies after 4-5 d. In all cases viability was greater than 50%. The Linbro tissue culture dishes were swirled briefly to mix the amoebae, wrapped in foil, and incubated without shaking at 21 ± 1 °C for 7 d, after which the numbers of macrocysts were determined (Robson & Williams, 1979).

RESULTS

Macrocyt formation between TS12 (matA1) and V12 (mata2) grown on 0.1% LP agar. Figure 1 shows the results of four experiments in which amoebae of strains TS12 (NC4-derived, matA1) and V12 (mata2) were grown on 0.1% LP agar in the light or the

![Graphs showing macrocyt formation](image-url)
dark and then harvested, washed, and placed in 1 ml 20 mM-CaCl₂ in the dark. In each
treatment one strain was present at a concentration of 5 × 10⁶ amoebae ml⁻¹ and successive
dilutions of the other strain were set up. Eight treatments are shown, grouped in pairs to
accentuate the effect of prior growth in the light versus the dark, while other variables are held
constant. For example, Fig. 1(a) shows the effect of 5 × 10⁶ amoebae of strain V12 (mata2),
grown in the light or the dark, mixed with dilutions of dark-grown strain TS12 (matA1). The
number of macrocysts formed when both strains were at high cell density was not
significantly different in the four treatments tested (i.e. light TS12 and V12; dark TS12 and
V12; dark TS12 and light V12; dark V12 and light TS12). There was considerable
quantitative variation between experiments; this may have been due to differences in the
potential of cells to form macrocysts at the time of harvesting. However, in all four
experiments identical qualitative results were obtained. The most striking result was the
virtual absence of macrocysts following any treatment in which 5 × 10⁶ light-grown cells of
either strain V12 (Fig. 1a, b) or strain TS12 (Fig. 1c, d) were mixed with 5 × 10⁵ cells of
opposite mating type. In this situation it mattered little whether or not the cells at lower
density were grown in the dark or the light. By contrast, all treatments in which 5 × 10⁶
dark-grown cells of either mating type were incubated with 5 × 10⁵ cells of opposite mating
type produced many macrocysts.

The experiments shown in Fig. 1 were repeated using amoebae grown on SM agar, and the
same qualitative results were obtained, although fewer macrocysts were formed. It is likely
that the lowered production of macrocysts by cells grown on SM agar was due to the
presence of high levels of phosphate, a known inhibitor of macrocyst formation (Nickerson &

Macrocyst formation between low numbers of amoebae of one mating type and excess cells
of opposite mating type. We wished to establish whether single amoebae were capable of
zygote formation. Since macrocysts are formed by engulfment of large numbers of amoebae,
very low numbers of amoebae (50) of one mating type were mixed with a large excess of
amoebae of the other mating type. When 50 dark-grown V12 (mata2) amoebae were mixed
with excess dark-grown TS12 (matA1) amoebae (Fig. 1c), 10–50 macrocysts were formed,
whereas very few if any macrocysts were formed until 500 dark-grown TS12 (matA1)
amoebae were present with excess dark-grown V12 (mata2) amoebae (Fig. 1a). These
experiments have been repeated a number of times with different strains, with the same result.
In particular, a strain with the V12 mating type (mata2) but linkage groups II, III, IV, part of
I, and possibly VII derived from NC4 (the matA1 strain) still formed 30 macrocysts when 50
amoebae were mixed with 5 × 10⁶ amoebae of matA1 mating type (D. Welker, personal
communication). This suggests that the effect is likely to be mating-type specific, rather than
being related to the genetic background of the strain.

DISCUSSION

The techniques used here differ in three ways from most previous studies on macrocyst
formation in D. discoideum. Firstly, the strains of opposite mating type were grown
separately; secondly, the assay involved placing washed cells in nutrient-free liquid where
asexual fruiting body formation was prevented; and thirdly, the ratios of the two cell types
were varied, to determine the effect of cell density.

Light-grown cells incubated together at high density in the dark in liquid formed as many
macrocyts as dark-grown cells incubated in the dark, in contrast to the predictions of Erdos
et al., (1976), who observed asexual fruiting bodies and no macrocysts when cells were grown
for only 24 h in the light before being transferred to the dark. We propose that Erdos et al. 
(1976), who suggested that cells become committed very early in the growth phase to either
macrocyst development or asexual fruiting body formation, were actually measuring the
effect of light on a switch to one or other developmental pathway, rather than the ability to
undergo a particular development. It is clear that cells are not fully committed to either macrocyst formation or asexual fruiting body formation during the growth stage. However, it is only when cells are mixed at high density that light during the growth phase has no effect on macrocyst production. Presumably other factors limit the maximum number of macrocysts formed under these conditions. One such factor may be the territory size of each zygote. Fukui (1976) observed in Dictyostelium mucoroides that zygotes seek out other zygotes and engulf them. Thus above a critical density of zygotes, cannibalism may limit the maximum numbers of macrocysts formed.

That light does affect growth-phase amoebae, leading to decreased macrocyst production, was demonstrated in experiments in which the cell density was varied. While many macrocysts were formed when excess dark-grown cells of either mating type were mixed with few cells of the opposite mating type, very few macrocysts were formed when excess light-grown cells were mixed with few cells of opposite mating type. This is clear evidence for a light-sensitive step in the pathway leading to macrocyst formation for strains of both mating types. Using slightly different conditions (in particular, cells were incubated in the light at high density during macrocyst formation) O'Day & Lewis (1981) have obtained evidence for a light-sensitive step in the maturation of the mata strain (V12) but not the matA strain (NC4).

Our quantitative studies show that most amoebae can participate in zygote formation but that the two mating types differ in their capacity to participate. When as few as 50 dark-grown mata amoebae were mixed with $5 \times 10^6$ dark-grown matA amoebae, significant numbers of macrocysts were formed, indicating that probably any mata amoeba can participate in zygote formation. By contrast, about 500 dark-grown matA amoebae were required before any macrocysts were observed after mixing with $5 \times 10^6$ dark-grown mata amoebae. These results are similar to those of Lewis & O'Day (1979), who suggested that a hierarchical mating system exists in D. giganteum. They showed that the ability of one strain to induce macrocyst formation in another strain is inversely related to its ability to respond to an inducing stimulus. In these terms NC4 (matA1) is a strong inducer and poor responder, while V12 (mata2) is a poor inducer but strong responder. This represents evidence for two-way induction in D. discoideum macrocyst formation.

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**REFERENCES**


