

## Choice of Partners: Sexual Cell Interactions in *Dictyostelium discoideum*

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**ABSTRACT.** Recognition of mating partners is of central importance in the sexual processes. In consideration that the most important function of sexuality is to shuffle genetic materials to generate wider variation of characters, mating among different genetic backgrounds is preferable. Wild isolates of cellular slime mold *Dictyostelium discoideum* are predominantly heterothallic, but homothallic ones also exist. In addition, there are bisexual strains which are compatible with either mating type of heterothallic strains but are self-incompatible. How cells of these organisms choose proper mating partners may include the essential mechanisms for sexual cell recognition in general. This minireview addresses studies on sexual cell interactions of *D. discoideum* with special attention to cell recognition and evolution of the mating system.

### A. Introduction

Nearly all living organisms known today possess sexual systems, including those which predominantly propagate by asexual reproduction. Sexuality generates broader spectra of characters by shuffling genetic materials, and exerts profound advantages for the survival of species in variations of environmental conditions (1). Accordingly, mating should occur between different genetic backgrounds to maximize the benefit of sexuality and to meet the cost of sexual reproduction. In this sense, heterothallism (mating with non-self) is preferable to homothallism (mating with self). Higher organisms are mostly heterothallic, having two complementary mating types. How was sex invented, and how did the sexual reproduction system evolve? What are the molecular bases for the proper choice of mating partners? What are the best ways to approach to these questions?

In this minireview, I will survey studies on the sexual system of the cellular slime mold *Dictyostelium discoideum* with attention to cellular recognition during mating. This organism has many useful properties as an experimental material. It is haploid during most of its life cycle, with small genome size (about 35 Mb), proliferates as single amoebae with a generation time as short as 3 to 5 h while maintaining potentiality to construct multicellular structures, and can be genetically engineered with ease. With respect to sexual studies, the induction of sexual maturation is under experimental control, and synchronous cell fusion can be achieved (2). In

consideration that the sexual systems of cellular slime molds are primitive and apparently exhibit a prototypical sexual system, their analysis may provide some hints for the above questions.

### B. Asexual fruits or sexual cysts: developmental fate in cellular slime molds

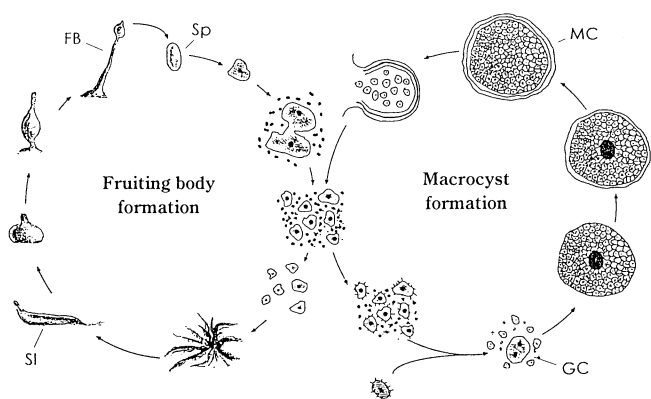
The cellular slime molds are known for their unique unicellular-multicellular asexual cycling and for their potential as excellent model organisms for multicellular development. However, less is known about their sexual life cycle, which is also unique, interesting, and suggestive.

Sexual and asexual life cycles of *Dictyostelium discoideum*, the most widely used cellular slime mold species, are shown in Figure 1. Unicellular amoeboid cells usually feed on bacteria and propagate by binary fission. Upon starvation, they gather and form multicellular structures called slugs, migrate for better location (toward light and away from ammonia), and make up fruiting bodies in which cells are differentiated into spores and stalk cells. Spores germinate when dislocated to fertile places. Alternatively, when the environment is dark and flooded, the amoeba sexually mature, and, in the presence of proper mating type cells, they fuse together to form zygotic cells called giant cells. The giant cells secrete cAMP and attract surrounding cells by chemotaxis, engulf and digest them, and finally develop into macrocysts (3, 4). After weeks of dormancy, macrocysts germinate to yield offspring amoebae (5).

### C. Polymorphic mating systems

The first description of macrocysts was in 1957 (6), and nearly two decades were necessary to recognize that

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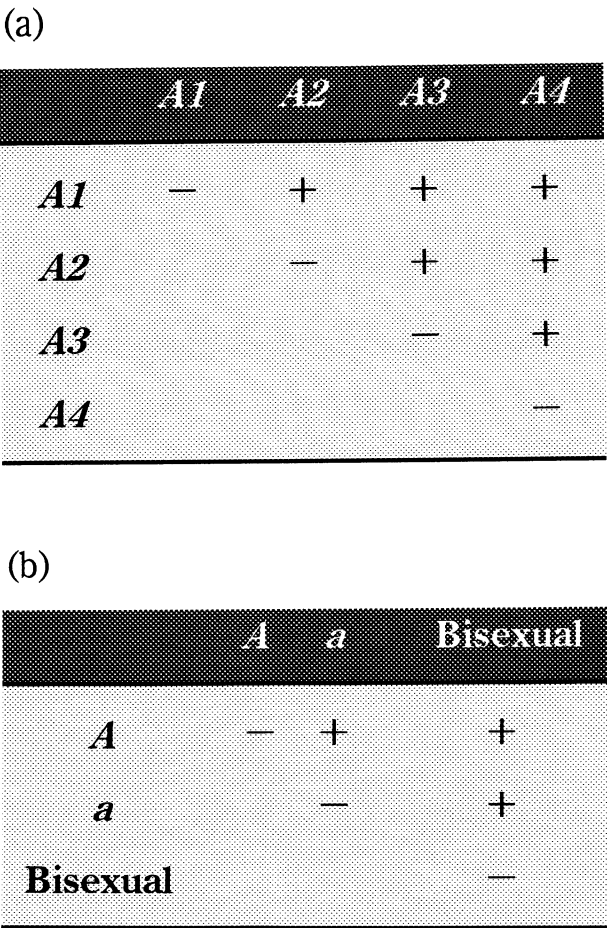


**Fig. 1.** Life cycles of the cellular slime mold *Dictyostelium discoideum*. Proliferating amoeba (center) can start either asexual development to form fruiting bodies (left circle) or sexual development to form macrocysts (right circle). FB: fruiting body; Sp: spore; Sl: slug; MC: macrocyst; GC: giant cell.

they were products of the sexual process (7). As a natural consequence of the fact that cellular slime molds mainly propagate asexually and, therefore, the local populations are mostly clonal, homothallic mating was detected first. Later systematic studies revealed the existence of heterothallic strains in a variety of species (8, 9, 10, 11).

Mating systems of several widely-used species of cellular slime molds are summarized in Table I. Most of the species are heterothallic, having two complementary mating types (the binary system). An exception, *Dictyostelium giganteum* possesses four mating types that comprise a multiple mating system, which is typical to some protozoa such as *Tetrahymena* (12). In this multiple mating system, strains of one mating type can mate with those of all other mating types, but cannot mate within the same mating type (Fig. 2a).

The mating system of *D. discoideum* is polymorphic.

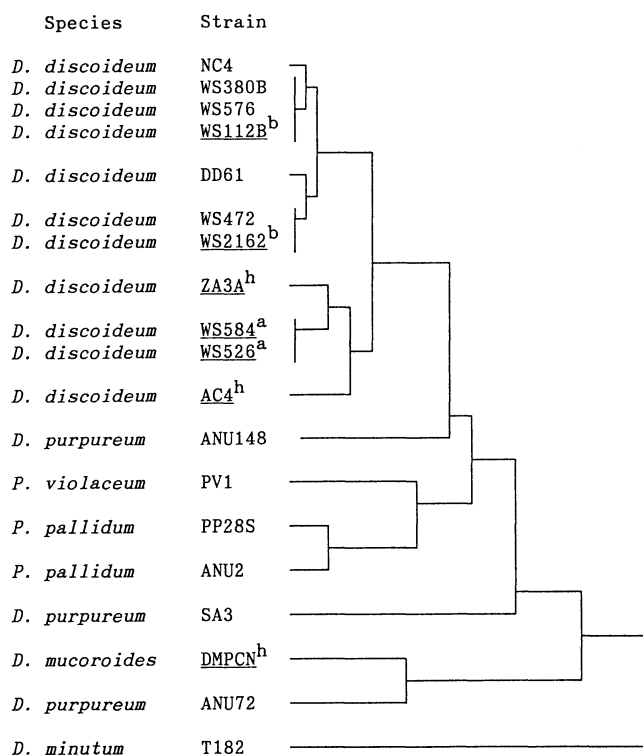


**Fig. 2.** Two heterothallic mating systems in cellular slime molds. The multiple mating system (a) is observed in *D. giganteum*, and the binary mating system with bisexual strains (b) is in *D. discoideum*. + and — represent compatibility and incompatibility of mating, respectively.

**Table I.** MATING SYSTEMS IN CELLULAR SLIME MOLDS

Species	Mating system (mating type)	References
<i>D. discoideum</i>	Heterothallic ( <i>mat A</i> , <i>mat a</i> )	Erdos <i>et al.</i> , 1973 (8)
	Homothallic	Robson and Williams, 1980 (13)
	Bisexual	Erdos <i>et al.</i> , 1973 (8)
	Asexual	Robson and Williams, 1980 (13)
<i>D. purpureum</i>	Heterothallic ( <i>mat I</i> , <i>mat II</i> )	Clark <i>et al.</i> , 1973 (9)
<i>D. giganteum</i>	Heterothallic ( <i>A1</i> , <i>A2</i> , <i>A3</i> , <i>A4</i> )	Erdos <i>et al.</i> , 1975 (11)
<i>D. mucoroides</i>	Homothallic	Weinkauff and Filosa, 1965 (30)
<i>P. pallidum</i>	Heterothallic ( <i>mat I</i> , <i>mat II</i> )	Francis, 1975 (10)
<i>P. violaceum</i>	Heterothallic	Clark <i>et al.</i> , 1973 (9)

In the homothallic mating system, cells of one strain can mate each with the other to form macrocysts within the clonal populations, while cells of a heterothallic strain require coexistence of different mating type cells. When a strain of a heterothallic species with two complementary mating types can mate with strains of either mating type, it is called bisexual.



**Fig. 3.** A phylogenetic tree of the slime molds based on allozyme polymorphism (14). Relative distances are shown. All strains are heterothallic except for underlined and marked. a: asexual; b: bisexual; h: homothallic.

The majority of this species are heterothallic, but a few of the strains are known to undergo homothallic mating. Some strains of *D. discoideum* form macrocysts with heterothallic strains of either mating type, and are called bisexual strains (Fig. 2b). Since they do not self-mate, we may regard them as the third mating type, *D. discoideum* exhibiting a multiple mating system with three mating types. Asexual strains have not been observed to form macrocysts and are considered to be defective mutants (13).

Why is the mating system of *D. discoideum* thus polymorphic? Several years ago, when molecular approaches to phylogeny became popular, the morphol-

ogy-based classification of slime mold species was reconsidered. According to polymorphisms of isozymes (14) and of restriction fragment length (15), the assignment of homothallic and asexual strains to *D. discoideum* was argued to be inappropriate. In a phylogenetic tree thus developed, homothallic and asexual species of *D. discoideum* are on a distinct branch from that of heterothallic ones, whereas bisexual strains rest on the same branches with typical heterothallic strains of *D. discoideum* (upper portion of Fig. 3). If we define species standing on mating compatibility, this argument fits well, because heterothallic and bisexual strains are mutually cross-matable, but there is no evidence of mating between homothallic and heterothallic strains of *D. discoideum*.

Since no other species of cellular slime molds exhibit mating polymorphisms, the assignment of homothallic and asexual strains to distinct species seems to be acceptable. Yet we should keep in mind that homothallic and asexual "*D. discoideum*-like" strains are closer to typical (heterothallic) *D. discoideum* strains than those of other species. The variation of mating systems within single or among closely related species is intriguing in consideration of evolution of the sexual system.

#### *D. Molecules involved in sexual cell interactions*

What kind of molecules are involved in the sexual cell interactions? The mechanisms of sexual cell interactions in *D. discoideum* have been investigated using the heterothallic strains NC4 (*mat A*) and HM1 (*mat a*). At an early stage of the molecular analysis of cell fusion mechanisms, fusion-inhibiting antibodies were used to identify fusion-related molecules. Such molecules are listed in Table II. Two of them, gp70 and DE1 antigen, are mating-type specific and probably responsible for partner recognition. The former, which has been shown to function in a  $Ca^{2+}$ -dependent manner (16), is specific to *mat a*, and the latter, to *mat A*. Others are common to both mating types and are suspected to be involved in the step of membrane fusion.

Among the fusion-related proteins, gp138 has been studied most extensively. Two genes which can encode gp138, *GPI38A* and *GPI38B*, have been cloned (17).

**Table II.** CELL FUSION-RELATED PROTEINS IN *D. discoideum*

Protein	Molecular mass (kDa)	Specificity	Reference
gp70	70	<i>mat a</i>	Urushihara <i>et al.</i> , 1988 (31) Ishikawa <i>et al.</i> , 1990 (16)
gp138	138	Nonspecific	Suzuki and Yanagisawa, 1989 (32) Suzuki and Yanagisawa, 1990 (33)
DE1 antigen	23	<i>mat A</i>	Aiba <i>et al.</i> , 1992 (34)
GG6 antigen	32, 125	Nonspecific	Aiba <i>et al.</i> , 1992 (34)
HH9 antigen	60	Nonspecific	Aiba <i>et al.</i> , 1992 (34)

They are mutually very similar with identity of 91.8% in the nucleotide sequence, but their temporal expression patterns are reversed; namely, *GP138A* is expressed in sexually mature cells and *GP138B*, in immature cells. The expression of antisense RNA of *GP138B* repressed the sexual cell fusion as well as the expression of gp138, proving the critical importance of *GP138A* and/or *GP138B* in sexual cell fusion (18). The gp138 protein is currently regarded as a cell-adhesion molecule for the following two reasons. First, its deduced amino acid sequence is partly similar to gp80, an EDTA-resistant cell adhesion protein during fruiting body formation in *D. discoideum*. Second, the nucleotide sequence of *GP138B* is the same as that of gp130, which functions in EDTA-sensitive cell adhesion in this species (19). Our very recent study indicated that an additional gene for gp138 exists (20). The origin and structural and functional relationships of multiple gp138 genes are interesting but still elusive problems.

#### E. Mechanisms of partner choice in *D. discoideum*

Ligand-receptor type molecular interactions have been reported for partner choice in several organisms. These interactions include mating pheromones and their receptors in yeast (21) and *Euprotes* (22), plus- and minus-agglutinins in *Chlamidomonas* (23), and fertilin (24) and  $\alpha 6\beta 1$  integrin (25) in mammals. Heterothallic strains in *D. discoideum* are also thought to recognize mating partners by such interactions. Supposing that *mat A*- and *mat a*- substances are responsible for mutual recognition, what kind of mating-type substances do bisexual strains possess? Since they accept both *mat A* and *mat a* cells as mating partners, they probably possess *mat a* and *mat A* substances as well. Nevertheless,

they are self-incompatible. Therefore, bisexual strains must have the means to discriminate self and non-self underlying the self-incompatibility.

Self-incompatible and bisexual fusion can be explained by two alternative models (Fig. 4) (26). In the "imperfect" model, surface molecules involved in cell fusion are defective in bisexual cells, but these defective molecules can interact with functional receptors on *mat A* or *mat a* cells. The alternative "self-recognition" model postulates the existence of an additional molecule on the surface of bisexual strains to recognize self. Its homophilic interaction is suggested to interfere with the function of cell fusion molecules. According to the self-recognition model, bisexual strains are potentially self-competent. Although these two models have the same end results, they are opposite in causality; the former postulates the loss of function, and the latter, the gain of a new function.

Recently, we found that a mild protease treatment of the cell surface of a bisexual strain rendered it self-compatible (27). Namely, cells of a bisexual strain WS2162 which are competent for cell fusion with heterothallic strains of either mating type but incompetent with self, can fuse with themselves after trypsin treatment of their surfaces. This fact indicates that there is a self-recognition molecule which inactivates cell fusion mechanisms on the surface of bisexual strains, supporting the self-recognition model of self-incompatibility. Another interesting finding is that the protease-induced self-fusion products of the bisexual strain cannot complete macrocyst development. Therefore, a two-check-point system may operate to ensure self-incompatibility of bisexual strains.

#### F. Prospects

*D. discoideum* is a superb model organism as described earlier. In addition, a new technique called REMI (Restriction-Enzyme-Mediated Integration) mutagenesis (28, 29) was invented using this organism. REMI is an efficient method of insertional mutagenesis which allows rapid isolation of functionally important genes. However, in consideration of the rapid progress in experimental techniques, simply being convenient experimental materials may not be a great advantage. Rather, the importance of cellular slime molds is that they have unique mating systems. For example, polymorphic mating systems will be valuable in order to trace the evolution of sexuality; bisexuality may be an intermediate form of transition from homothallism to heterothallism, which is critically important for sexual evolution. The self-recognition model of bisexuality suggests that the invention of self-recognition molecules may have been the starting point for heterothallism. Thus, our understanding of the mechanisms of sexual cell fusion in these simple organisms will clarify how

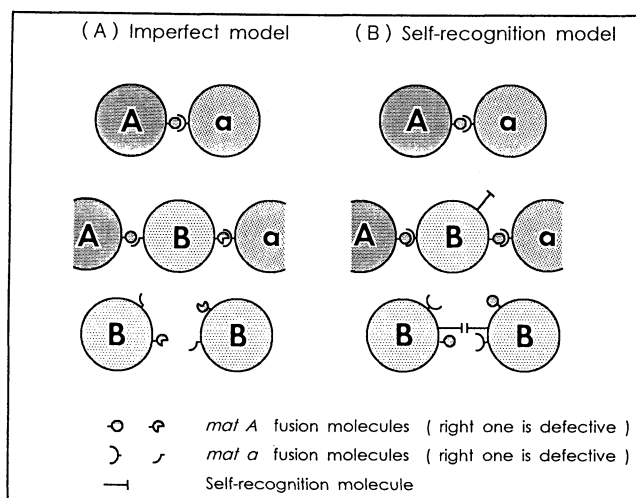


Fig. 4. Two simplified models for self-incompatible and bisexual mating (26). A: *mat A* cell; a: *mat a* cell; B: bisexual cell.

cell recognition systems in general and sexual systems evolved.

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