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—Review—
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The Cellular Slime Mold: Eukaryotic Model Microorganism

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Abstract: Cellular slime molds are eukaryotic microorganisms in the soil. They feed on bacteria as solitary amoebae but conditionally construct multicellular forms in which cell differentiation takes place. Therefore, they are attractive for the study of fundamental biological phenomena such as phagocytosis, cell division, chemotactic movements, intercellular communication, cell differentiation, and morphogenesis. The most widely used species, *Dictyostelium discoideum*, is highly amenable to experimental manipulation and can be used with most recent molecular biological techniques. Its genome and cDNA analyses have been completed and well-annotated data are publicly available. A larger number of orthologues of human disease-related genes were found in *D. discoideum* than in yeast. Moreover, some pathogenic bacteria infect *Dictyostelium* amoebae. Thus, this microorganism can also offer a good experimental system for biomedical research. The resources of cellular slime molds, standard strains, mutants, and genes are maintained and distributed upon request by the core center of the National BioResource Project (NBRP-nenkin) to support *Dictyostelium* community users as well as new users interested in new platforms for research and/or phylogenic consideration.
Key words: cell differentiation, chemotaxis, *Dictyostelium discoideum*, human genes, pathogen infection

Introduction: Why Microorganisms?

The cellular slime moulds, or dictyostelids, are soil microorganisms that exhibit conditional multicellularity accompanied with cell-type specialization [9]. Because of this unique property, they are often called "social amoeba". Such microorganisms might be considered to have little to do with experimental animals, but this is not appropriate. If we think about *Escherichia coli*, no one would disagree with its enormous contribution to biological research both as a model system to analyze the basic mechanisms of life and as a useful tool for molecular cloning. Likewise, dictyostelids can offer a remarkable experimental system especially valuable for eukaryote-specific processes in which *E. coli* is not involved.

The life cycle of the major dictyostelid, *Dictyostelium discoideum*, is shown in Fig. 1. The growth phase cells actively phagocytose bacteria as food and undergo cell division. After consumption of all the surrounding bacteria around, starvation triggers aggregation of cells and their transformation to multicellular pseudoplasmodia, which finally develop into fruiting bodies composed of spore balls and supportive stalks. This process is called

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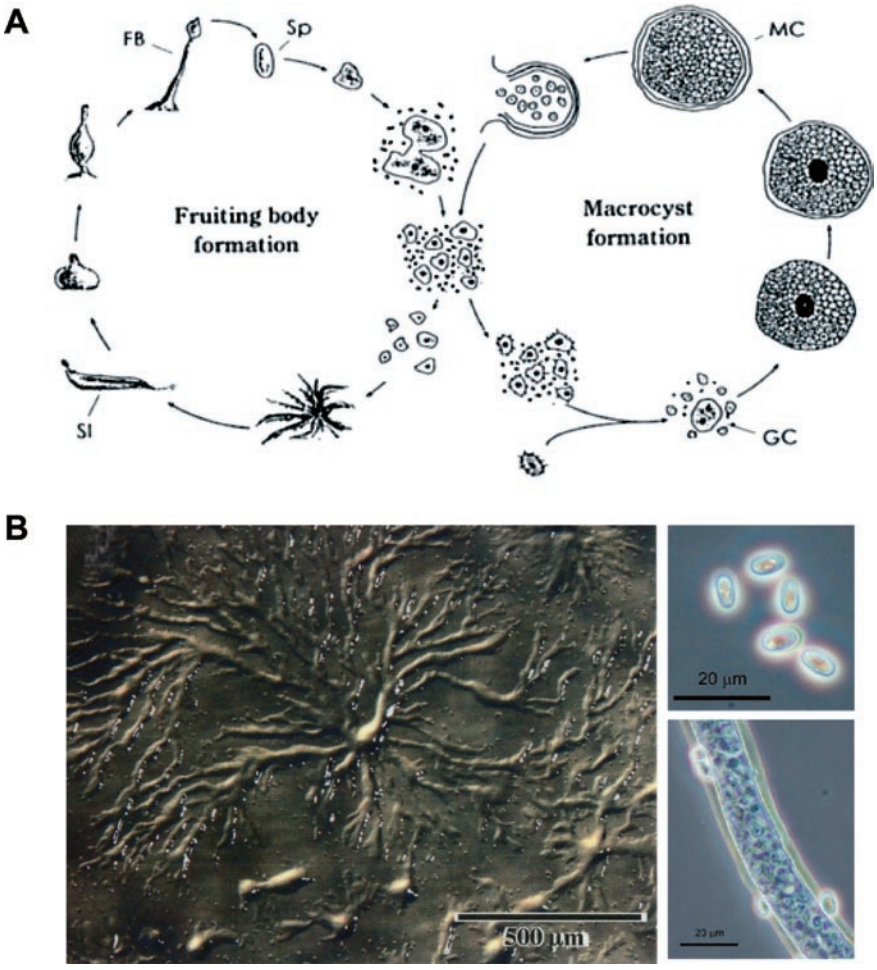

Fig. 1. (A) Schematic illustration of the life cycle of *D. discoideum*. Growth phase cells initiate asexual development (left circle of top) upon starvation. Under dark and submerged conditions, they start sexual development (right circle of top). Sl; slug (pseudoplasmodium), FB; fruiting body, Sp; spore, GC; giant cell (zygote), MC; macrocyst. (B) Pictures of aggregating cells (left), spores (right top), and stalk cells (right bottom) are shown.

asexual development. Under dark and submerged conditions, the cells become sexually mature, fuse with appropriate mating-type cells, and develop into dormant structures called macrocysts to complete the sexual development. This life cycle of *D. discoideum* makes it attractive for the study of basic biological phenomena such as phagocytosis, cell division and movement, intercellular communication, cell differentiation and morphogenesis. In addition, *D. discoideum* cells are amenable to laboratory handling, experimental systems for cell culture and synchronous development having been established. A large quantity of cells can be obtained without much labour, and all kinds of molecular biological techniques have been successfully applied to them. *D. discoideum* cells can be used as a platform for analyzing the function of foreign genes [5]. Thus, NIH has cited *D. discoideum* as a non-mammalian model organism for biomedical researches (http://www.nih.gov/science/models/).

Standard Strains and Their Properties

Genus, species, and strains

Three genera, *Dictyostelium*, *Polysphondylium*, and

Acytostelium, are described as dictyostelids. They can be distinguished mainly by their stalk morphology (i.e., branched or unbranched, cellular or acellular). The dominant trend in evolution seems to be increased size and cell-type specialization, and *D. discoideum* is in the most advanced group [17]. Two complementary mating-type strains in *D. discoideum*, NC4 (*mat I*) and V12 (*mat II*), have been used as standard strains because of their stable development and ease of use in experimental manipulation. Although those standard strains grow on bacteria as well as wild isolates, some of the mutant strains like AX2 and AX3 originated from NC4, and their derivatives, can grow in nutrient liquid media without bacteria (axenic media) due to their higher activities of macropinocytosis. Since this property is especially valuable for biochemical and molecular biological studies, the axenic strains are widely used, today, in place of NC4. In addition to the standard strains of *D. discoideum*, standard strains of other major species such as *Dictyostelium mucoroides*, *Polysphondylium pallidum*, and a wide range of spontaneous, chemically induced, insertional, and designed mutants are available.

Growth and development

D. discoideum cells are haploid throughout growth and asexual development. Only for a short period during the sexual process, do they exist as diploid cells. The doubling time of growth phase cells is approximately 3 h in a 2-member culture on nutrient agar plates with *E. Coli* B/r or *Klebsiella aerogenes*, and 8 h in a standard axenic medium at 22°C. The cool circumstances are favourable for the dictyostelids and the cells normally do not survive temperatures higher than 27°C. If grown on nutrient agar plates with food bacteria, 1–5 × 10^9 cells/dish (9 cm diameter) can be obtained before starvation. Since the starved cells immediately enter into the process of fruiting body formation and generate dormant spores, the cultures can be left for weeks without care. The 2-member culture with bacteria is thus a convenient way for strain maintenance. Moreover, it guarantees that the genome is healthy enough to support normal asexual development.

Molecular biological techniques

Transformation of *Dictyostelium* cells is carried out by electroporation. Clonal lines of transformants can be established normally in 3 weeks or so. Drug resistance markers such as *neo*^{r} (G418 resistance) and *bsr* (blasticidin S resistance) work well as long as axenic strains are used. Bacterially grown cells do not give clear results of selection. Since homologous recombination occurs effectively in *D. discoideum*, targeted gene disruption does not require a negative selection procedure. High-copy number plasmids were found in wild isolates of *D. discoideum* and were used to construct the shuttle vectors that extrachromosomally replicate both in *E. coli* and *D. discoideum*. Almost all recent molecular biological techniques have been successfully applied to *D. discoideum* [e.g., 12,14].

Genome information

The genome analysis of *D. discoideum* was published in 2005 [3]. It has an (A+T)-rich (78.8%) genome of approximately 33.8 Mbp in size and with around 12,500 genes. Molecular phylogeny studies indicate that the dictyostelid lineage branched after plants and before fungi in the eukaryote tree (Fig. 2), yet it contains more genes related to human disease than yeast does. Among 287 protein sequences for confirmed human disease genes, 64 have putative orthologues ($e \leq 10^{-40}$) in *D. discoideum*. Of these, 33 are highly homologous (similarity region ≥70% of the length) (Table 1). The EST analysis was performed by the Japanese cDNA Project (http://dictycdb.biol.tsukuba.ac.jp/) and the results have been made public as the *Dictyostelium* cDNA Database (Dicty_cDB). More than 150,000 reads from nearly 90,000 clones are clustered into approximately 7,000 UniGene sets [19, 20], about half of which cover the entire coding sequences. The genome and cDNA data are well annotated and publicly available at dictyBase and Dicty_cDB (Table 2).

How to Obtain and Handle the Resources

NBRP-nenkin

The cellular slime mold resources are maintained by the Core Center of National BioResource Project (NBRP-nenkin) (http://nenkin.lab.nig.ac.jp/en_top). University of Tsukuba and the Advanced Industrial Science and Technology (AIST) collaborate in this activity. Cur-

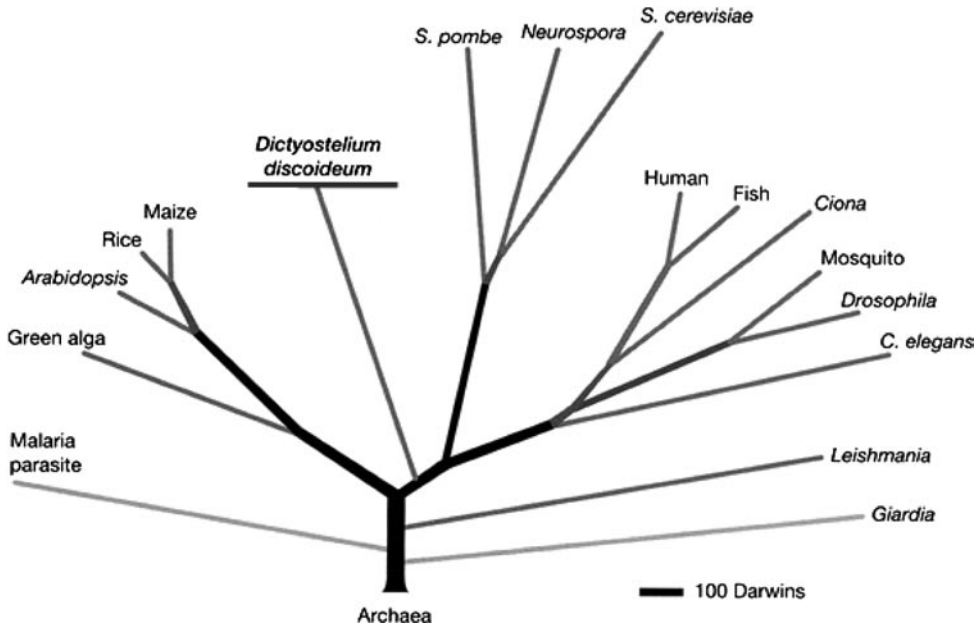

Fig. 2. A phylogenic tree showing the position of *Dictyostelium discoideum*. Reproduced from [3].

Table 1. Human disease genes with close orthologues in *Dictyostelium*

Disease category		Gene
Cancer	Colon cancer	*MLH1, MSH2, MSH3, PMS2*
	Xeroderma pigmentosum	*ERCC3, XPD*
	Oncogene	*AKT2, RAS*
	Other	*CDK4*
Neurological	Lowe oculocerebrorenal	*OCRL*
	Miller-Dieker lissencephaly	*PAF*
	Adrenoleukodystrophy	*ABCD1*
	Angelmann	*UBE3A*
	Ceroid lipofuscinosis	*CLN2, PPT*
	Tay-Sachs	*HEXA*
	Thomsen myotonia congenita	*CLCN1*
	Choroideremia	*CHM*
	Amyotrophic lateral sclerosis	*SOD1*
	Parkinson's	*UCHL1*
Cardiovascular	Hypertrophic cardiomyopathy	*MYH7*
Renal	Renal tubular acidosis	*ATP6B1*
	Hyperoxaluria	*AGXT*
Metabolic/endocrine	Niemann-Pick type C	*NPC1*
	Hyperinsulinism	*ABCC8*
	McCune-Albright	*GNAS1*
	Pendred	*PDS*
Haematological/immune	G6PD deficiency	*G6PD*
	Chronic granulomatous	*CYBB*
Malformation	Diastrophic dysplasia	*SLC26A2*
Other	Cystic fibrosis	*ABCC7*
	Darier-white	*SERCA*
	Congenital chloride diarrhoea	*DRA*

Modified from Eichinger *et al*., 2005 [3].

rently, University of Tsukuba handles cDNA resources and AIST strains. In terms of global community activity, the Dicty Stock Center (DSC) (http://dictybase.org/StockCenter/StockCenter.html) was established in 2003 at Columbia University. Although DSC has a wide range of resources deposited from the community members and its contribution is highly appreciated, we are aware of two problems: Japanese users have met some difficulties especially in transport of recombinant strains, and, just one resource center is dangerous in terms of accidents and discontinuation of resource grant. In order to solve these problems and to facilitate wider use of *Dictyostelium* resources in Japan, NBRP-nenkin was established in August 2007. NBRP-nenkin and DSC have agreed to mutually exchange unique resources whenever necessary.

Resources available from NBRP-nenkin

Strains: The strains ready for distribution from NBRP-nenkin are listed in the strain database (http://nenkin.lab.nig.ac.jp/en_strain). Since many more are being processed, consulting about unlisted strains by e-mail will be of help. As mentioned above, strains available at DSC are also available through NBRP-nenkin for Japanese users with only the cost for domestic transportation.

Genes: The representative clones of the UniGene sets generated by the cDNA Project were selected and are being rearranged in multi-well plates. Currently, generation of most of the full-length clones is complete and listed in the gene database (http://nenkin.lab.nig.ac.jp/en_gene). Rearrangement and integration to the database will be extended to the entire UniGene. Any clones in Dicty_cDB, even those not regarded as representative, can be requested.

Resource distribution

Requested resources are currently sent free of charge except for the cost of transportation. Although the cost of resource handling may have to be covered by the user in the future, it should not be very high. As a usual procedure, an exchange of Material Transfer Agreement will be asked.

The strain will be delivered either as a culture on a nutrient agar plate with bacteria or as a low-density culture in a tube of axenic medium. It is recommended to prepare spore stocks in silica gel or DMSO stock of amoebae before experimental use. Since the growth phase cells of *D. discoideum* are haploid and proliferate with a short doubling time, genetic variation quickly accumulates, resulting in heterogeneity of the population. Therefore, long-term continuation of the culture should be avoided.

The cDNA clones will be sent as plasmid DNA, instead of *E. coli* to avoid complicated procedures required for both donors (NBRP-nenkin) and recipients (users) to transport genetically modified organisms.

Starting the culture

Since one of the major purposes of NBRP-nenkin is to enhance the wider use of *Dictyostelium* as a research resource, assistance for new users not familiar with *Dictyostelium* is a high priority task. Consultation about resource handling will be welcome. If necessary, training courses for new users will be arranged. Detailed methods of cultivation and storage can be found in the literature [e.g., 21], but the essential protocol will be supplied upon request.

No additional equipment to start the culture of *Dictyostelium* is necessary for laboratories set up for tissue culture of animal cells. Clean bench, temperature-controlled incubators (without gas supply), microscope, and autoclave are the basic equipment. Although a –80°C freezer or a liquid nitrogen tank is necessary for storage of axenic strains, they are commonly used laboratory equipment.

Contribution of *Dictyostelium* to the Major Publications

cAMP signalling and chemotaxis

G-protein coupled cell surface receptors for cAMP play essential roles both for chemotactic cell aggregation and for cell differentiation during asexual development of *D. discoideum* [11]. Secretion of cAMP, which normally acts as an intracellular second messenger, is unique to the cellular slime molds but the intracellular signalling and outputs are common to a wide range of organisms [2]. The PTEN (Phosphatase and Tensin Homolog) -involved mechanism of polarity determination has been elucidated [6] and further studied at the single molecule

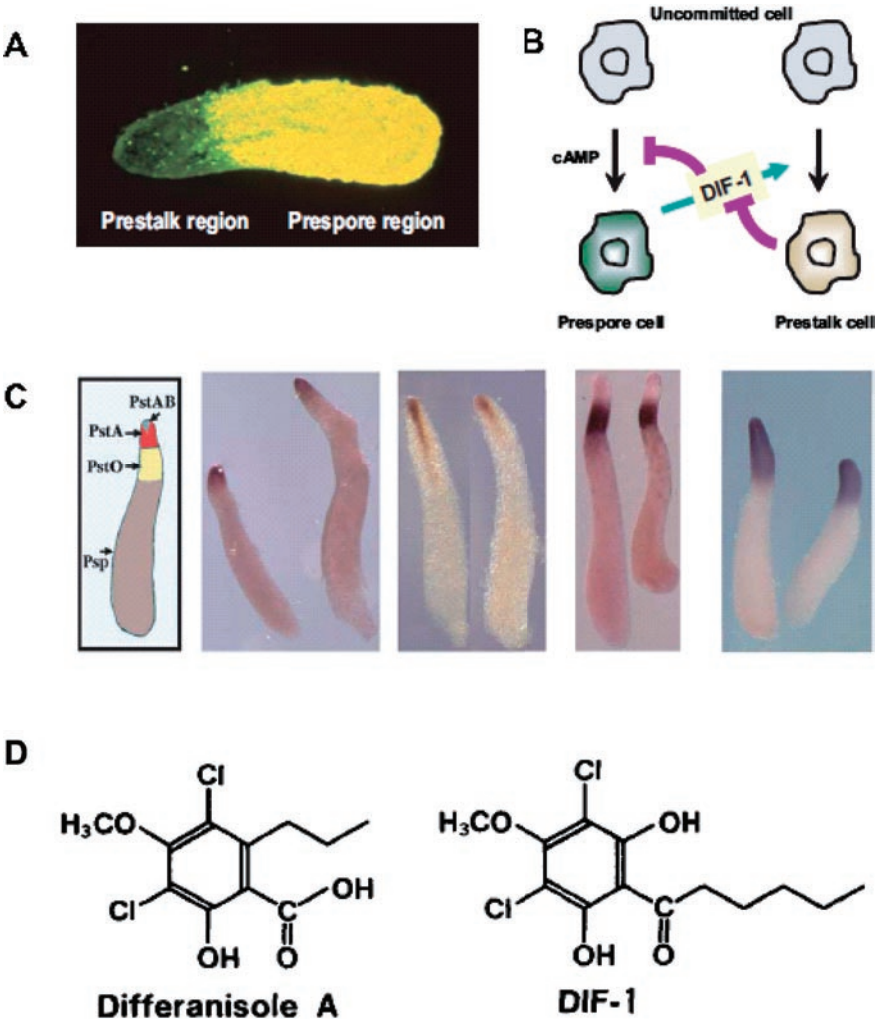

Fig. 3. Pattern formation in the slug of *D. discoideum*. (A) A slug was treated with anti-spore antibody. Posterior (right) 80% was stained. Courtesy of Dr. I. Takeuchi. (B) A feed-back loop of DIF-1 regulation. (C) Whole mount *in situ* hybridization showing sub-regions of the prestalk region characterized by differential gene expression. Courtesy of Dr. M. Maeda. (D) Chemical structures of the morphogen DIF-1 (major component of DIF) (right) and differanisole (left) are shown.

level [15]. *D. discoideum* has four cAMP receptors each with a distinct function [10]. How this signalling system evolved has recently been elucidated [16].

Morphogen signalling and cell differentiation

Cellular commitment either to spore or stalk-cell lineage is observed in a pseudoplasmodium as a clear pattern formation of prestalk and prespore regions (Fig. 3A). How cell-type specification occurs in the originally homogeneous population of cells is an intriguing issue. A chlorinated polyketide named DIF (Differentiation Inducing Factor) (Fig. 3D right) has been shown to act as a morphogen which induces cells to become the stalk-cell lineage [8]. A feed-back loop for generation and degradation of DIF is involved in this process (Fig. 3B). The STAT (Signal Transducer and Activator of Transcription) mediated regulation of gene expression is involved in a downstream cascade of DIF signalling to more detailed cell-type determination [22] as can be seen in Fig. 3C. It is worth noting that differanisole, (Fig. 3D left) which is used for medical treatment of human leukemia and is structurally very similar to DIF,

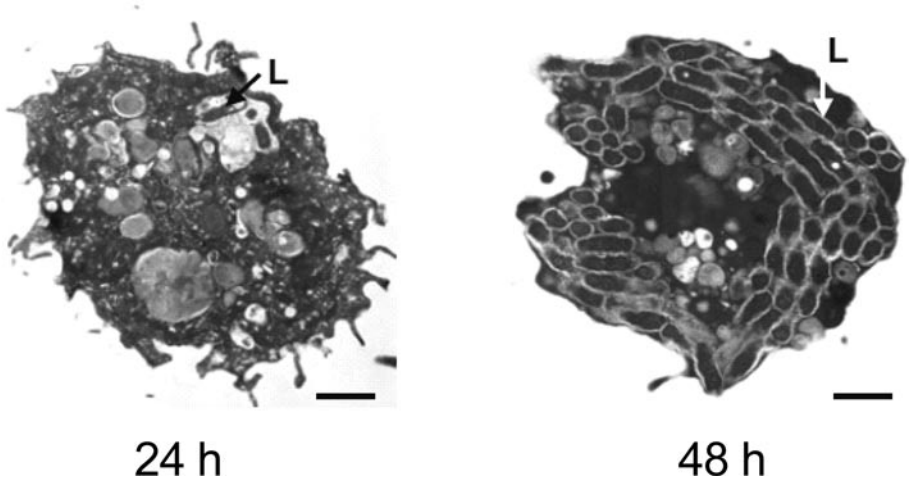

Fig. 4. Infection of *Legionella* to *D. discoideum* cells. L: *Legionella*. After 48h of infection, the entire cytoplasmic region is filled with proliferated *Legionella*. Bar; 10 μm. Reproduced from [4].

Table 2. Location of resource information

Name of site	URL	Contents
NBRP-nenkin	http://brc.gene.tsukuba.ac.jp/nenkin/	Strains, cDNA clones (Japanese site)
Dicty Stock Center	http://dictybase.org/StockCenter/StockCenter.html	Strains, plasmids, techniques
dictyBase	http://dictybase.org/	Central information resource
Dicty_cDB	http://dictycdb.biol.tsukuba.ac.jp/cDNAproject.html	cDNA database
Japanese community	http://www.glyco.is.ritsumei.ac.jp/csmkkk/	Anual meeting of Japanese community

can induce stalk-cell differentiation [13]. DIF in turn induces erythoid differentiation in leukemia cells [1].

Dedifferentiation

Dictyostelium exhibits a unique property: cells in developing multicellular structures rapidly dedifferentiate and restart proliferation when mechanically dispersed and food is supplied. This phenomenon is called an 'erasure' event and has been analyzed as a model for tissue regeneration. Microarray-based transcriptome analysis revealed that dedifferentiation is a genetically controlled process and at least one of the histidine kinases is involved in the process [7].

Interaction with pathogenic bacteria

Ever since the report of *Legionella pneumophila* infection of *D. discoideum* cells [18], analysis of cellular interaction with pathogenic bacteria has become a trend in *Dictyostelium*. In consideration of the fact that dictyostelids feed on bacteria, the properties of those bacteria should have a strong impact on cellular physiology. According to the results of transcriptome analysis of *D. discoideum* cells before and after infection, *L. pneumophila* inhibits expression of host genes required for membrane fusion between phagosomes, where the bacteria are retained, with lysosomes [4].

Where to obtain Dictyostelium information

The internet sites for *Dictyostelium* information are listed in Table 2. dictyBase is a central resource for the biology and genomics of *Dictyostelium* as described on its top page, whence almost all information can be obtained. It provides links to resource locations and explanation of cellular slime molds and techniques.

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