

Chapter 2

The Model Organism *Dictyostelium discoideum*

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Abstract

Much of our knowledge of molecular cellular functions is based on studies with a few number of model organisms that were established during the last 50 years. The social amoeba *Dictyostelium discoideum* is one such model, and has been particularly useful for the study of cell motility, chemotaxis, phagocytosis, endocytic vesicle traffic, cell adhesion, pattern formation, caspase-independent cell death, and, more recently, autophagy and social evolution. As nonmammalian model of human diseases *D. discoideum* is a newcomer, yet it has proven to be a powerful genetic and cellular model for investigating host–pathogen interactions and microbial infections, for mitochondrial diseases, and for pharmacogenetic studies. The *D. discoideum* genome harbors several homologs of human genes responsible for a variety of diseases, including Chediak-Higashi syndrome, lissencephaly, mucopolidosis, Huntington disease, IBMPFD, and Shwachman-Diamond syndrome. A few genes have already been studied, providing new insights on the mechanism of action of the encoded proteins and in some cases on the defect underlying the disease. The opportunities offered by the organism and its place among the nonmammalian models for human diseases will be discussed.

Key words *Dictyostelium*, Amoeba, *Legionella*, *Mycobacteria*, Chemotaxis, Actin, Cell motility, Phagocytosis, Host–pathogen interactions, Microbial infections, Shwachman-Diamond syndrome, SBDS, Huntingtin, Mitochondrial disease, IBMPFD, AMPK, Pharmacogenetics

1 Introduction

Among the so-called model organisms, i.e., the restricted number of organisms that have emerged as particularly suitable for cell and developmental biology studies, the cellular slime mold *Dictyostelium discoideum* (in the following *Dictyostelium*) is unique, due to cell division and development being totally uncoupled, and because of the transition from a unicellular to a multicellular stage during the life cycle (1). Growing cells proliferate by binary fission but do not differentiate, whereas starving cells undergo multicellular development and differentiation without dividing and without any need for external nutrients. Thus growth and development can be studied separately, and several nonlethal mutants can be isolated that

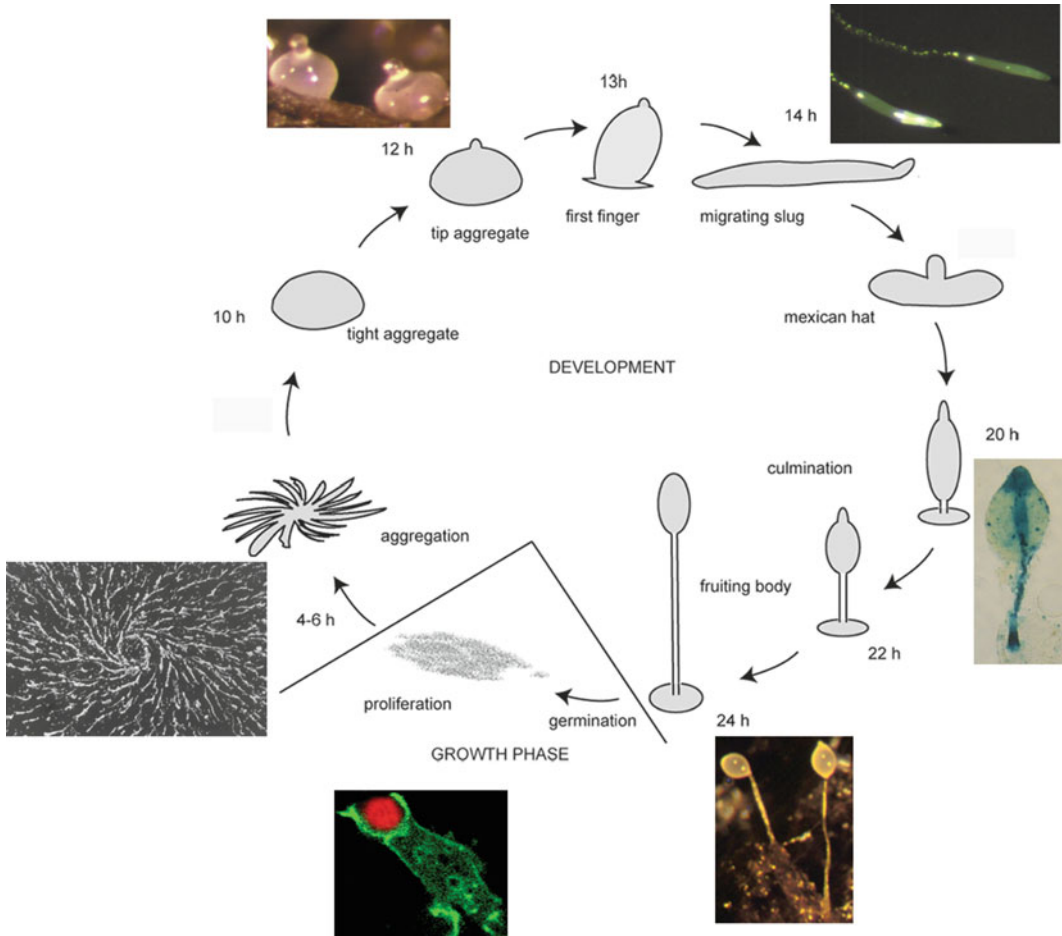


Fig. 1 Life cycle of *D. discoideum*. The life cycle of *D. discoideum* consists of two phases, growth phase and development. During growth, cells feed on bacteria and proliferate as unicellular amoebae by binary fission. Starvation triggers development, leading first to aggregation, then to transformation of tight aggregates into migrating slugs, and eventually to fruiting body formation. The different morphological stages with approximate timing on agar plate are depicted. A vegetative cell labelled with anti-actin antibody engulfing a yeast particle is shown at the *bottom*. The *dark grey labeling* in the stalk of the culminating fruiting body on the *right* is due to β -galactosidase expression under the control of a pre-stalk cell-specific promoter. See text for details

are affected in some aspects of development, while growing perfectly well. During growth and the initial stage of development up to formation of aggregates, the *Dictyostelium* colony is a population of single amoeboid cells that are capable of actively moving over solid substrata, ingesting bacteria by phagocytosis and proliferating up to depletion of their food source. In response to starving conditions, the cells develop the ability to gather together into aggregates by secreting and responding to the chemoattractant cAMP and by adhering to each other (Fig. 1). Several thousands of cells in each aggregate cooperate in constructing a migrating slug,

whereby the individual amoebae become integrated into a unitary sausage-shaped organism, the slug, coated by a secreted extracellular matrix. The slugs migrate over the substratum towards light and along temperature gradients (Fig. 1). Like animal embryos, each slug has an embryonic organizer—the anterior tip—that regulates collective behavior, cell fate, as well as final morphogenesis (1, 2). Roughly speaking, the post-aggregative stages of *Dictyostelium* life cycle are attractive for developmental biology studies, particularly for investigating the genetic basis of pattern formation and cell differentiation, whereas growth, pre-aggregation, and the aggregation stage are best recommended for cell biology studies.

The variety of cellular and developmental processes that can be easily studied in *Dictyostelium*, coupled with its genetic malleability, has made possible the development of a large armory of biological assays for analyzing mutants (3), which have not only led to its establishment as model organism for cell and developmental biology but have also favored its entry as nonmammalian model for biomedical research.

2 Experimental Virtues of *Dictyostelium* as Model Organism

Like other microbial organisms, *Dictyostelium* is cultured easily and cheaply, and can be frozen and stored indefinitely (4). It exhibits rapid growth (3–8-h duplication time, when cultured on bacteria or in axenic culture media, respectively), rapid development (24 h), and small size of mature organisms (micrometer range), making it possible to work with statistically high numbers of cells and organisms. Large yields of cells with defined identity can be easily cultured, facilitating biochemical studies. Growth and development occur at room temperature under atmospheric CO₂ levels, all stages of development can be easily followed on agar or on filter paper and, at least up to tight aggregate formation, even on a glass slide in a simple salt solution. Furthermore, a wide range of cell biological assays is available, including assays in cell motility and chemotaxis, phagocytosis and macropinocytosis, cell–cell and cell–substratum adhesion, resistance to osmotic stress, single cell differentiation, or cell death (3), just to mention a few. *Dictyostelium* cells were among the first eukaryotic cells in which in vivo imaging of fluorescent protein chimeras was applied (5), and they are amenable to any kind of imaging microscopy techniques. More importantly, for ease of genetic manipulation *Dictyostelium* is probably surpassed only by the yeast, although *Dictyostelium* differs from yeast in many respects, particularly regarding motility and multicellular development. A powerful collection of forward- and reverse-genetic tools has been worked out to (1) disrupt, replace, or silence a gene; (2) recover a mutated unknown gene; and (3) overexpress and/or tag a gene (3). *Dictyostelium* is haploid; therefore gene disruption

by homologous recombination usually causes phenotypes without the need for further manipulation (6). Multiple knockout mutants can be created by a Cre/LoxP-mediated recombination system (7, 8), thus facilitating analysis of gene families or gene networks. Recombination or complementation by mating is not possible, but mutants may be rescued by introducing the gene of interest in wild-type or mutated form. In addition to its use for disrupting genes by homologous recombination, random insertional mutagenesis by REMI (restriction enzyme-mediated integration) has been used for suppression genetics (9) or to create libraries containing random genetic insertions (10). Gene silencing by expression of antisense RNA or RNAi (11) as well as complementation of spontaneous or chemically induced mutants with cDNA libraries (12) have been shown to be possible, but their use has been so far limited.

All these genetic tools can be applied to a genome of 34 Mb, harboring by the most conservative estimate 10,300 genes (13, 14), compared to about 6,000 and 13,000 genes for yeast and *Drosophila* (15, 16), respectively. In addition to curated and annotated gene models that can be browsed in the *Dictyostelium* community resource database (www.dictybase.org), a cDNA library representing 55% of all genes expressed at different developmental stages is available (17). For a model organism it is also worth mentioning that a wide selection of plasmids, mutants, and stable cell lines with ablated or overexpressed genes is available in the *Dictyostelium* stock center (www.dictybase.org). Another important tool for experimental studies, namely, monoclonal or polyclonal antibodies against *Dictyostelium* cell antigens are unfortunately not commercially available, due to the relatively small size of the *Dictyostelium* community. However several hybridoma cell lines, which in the past were raised in different labs, are now stored and available in the Developmental Studies Hybridoma Bank (www.dshb.biology.uiowa.edu).

3 The Place of *D. discoideum* Among the Other Nonmammalian Model Organisms

Model organisms are by definition lower organisms in the evolutionary scale, in which for many practical reasons it is easy to study at the molecular level biological processes that are common across all, or at least several types, of organisms. From the remarks in the previous section it is clear that among the ten nonmammalian model organisms recognized by the NIH (www.nih.gov/science/models) *Dictyostelium* has several advantages as experimental organism. *Drosophila melanogaster*, *Caenorhabditis elegans*, *Arabidopsis thaliana*, or zebrafish can hardly be the first choice for biochemical or cell biological studies, due to limited availability of cell cultures with defined identity. The genetic gap with *Drosophila* has been overcome with the development of molecular genetics, and unlike yeast *Dictyostelium* displays multicellularity and cell

differentiation coupled with pattern formation and morphogenesis. On the other hand, development and physiology are clearly less complex in *Dictyostelium* than in *Drosophila*, *C. elegans*, or zebrafish, not to mention mice. It's rather unlikely that *Dictyostelium* could be a suitable model for, e.g., hematopoiesis or tumorigenesis, as *Drosophila* or zebrafish are, since there is neither blood nor cancer in *Dictyostelium* (patho)physiology, despite the cells harboring a STAT pathway (18, 19), and synthesizing a promising anticancer drug (20, 21). Therefore the question arises, for which biological processes has *Dictyostelium* proven to be a representative model organism? Although there are different levels for an organism to be a model (*D. discoideum*, e.g., is the leading genetic model of amoebozoa), it is trendy nowadays to consider lower organisms truly universal models, if they can be representative of, and thus modeling, mammals. Furthermore, the explicit or implicit assumption in any promotion of a model organism is that studies with the organism in question will provide insights and understanding of basic processes that are eventually relevant for human pathophysiology, and thus human diseases. This raises the second question on whether and to which extent *Dictyostelium* is a suitable model organism for biomedical research.

Before dealing with this question, it is worth mentioning all the biological processes that have been successfully modeled in *Dictyostelium*. It is well known that *Dictyostelium* is a leading model for eukaryotic chemotaxis (22–24) and one of established models for other motility-linked processes, such as cytokinesis (25), phagocytosis (26), macropinocytosis, and endo-lysosomal traffic (27, 28). With alternate success it has been and is also a model for cell–cell and cell–substratum adhesion (29–31), cell differentiation and pattern formation (32–34), and autophagy and apoptosis-independent cell death (35, 36). More recently, it has emerged as a powerful simple model for genetic analysis of social evolution (37, 38). These contributions have been reviewed extensively in the last years and the most recent reviews are listed in Table 1.

As expected for a good model, research done with *Dictyostelium* has produced relevant information, either by anticipating results and insights that boasted research in other organisms or by introducing methodological strategies later adopted in other systems. A few examples are the discovery of cAMP as the first chemoattractant long before the first chemokine was found in mammals (39, 40); the discovery that chemoattractant signals were transduced via G protein-coupled receptors (41); the role played by Ras proteins (42–45), AKT/PKB (46), PTEN (47, 48), and more recently by TORC2 (44, 49, 50) in chemotaxis, later confirmed also in mammalian cells (51–53); the immunological strategy for identifying cell–cell adhesion molecules (54–56), that was later adopted in mammals and led to the discovery of the N-CAM (57) and the first cadherin (58); the pioneering application of cryo-electron

Table 1**Biological processes for which *Dictyostelium* is an excellent model organism**

Biological processes	Most recent reviews
Motility and chemotaxis	(22–24, 159–164)
Phagocytosis, macropinocytosis, and endo-lysosomal traffic	(26, 28, 130, 165)
Cytokinesis	(25)
Cell–cell adhesion	(29)
Autophagy, non-apoptotic cell death	(35, 36, 166)
Development, pattern formation, quorum sensing	(167–170)
Social evolution	(37, 38, 168)

microscopy to intact eukaryotic cells (59) or of single-molecule force microscopy to measure cell–cell adhesion (60), later applied to leukocytes and neural cells, to N-CAM and cadherin (61–63); and the use of GFP-fused proteins for studying the in vivo dynamics of the actin cytoskeleton and intracellular vesicle traffic in phagocytosis (5, 64, 65) and chemotaxis (5, 66, 67), long before their use in macrophages (68) or neutrophils (69).

Dictyostelium has contributed significantly in identifying cytoskeletal and regulatory proteins of the mechanosensory system controlling cytokinesis (70–72). Similarly, investigation of autophagic cell death in the absence of apoptosis has been favored in this organism, because it displays developmental cell death, but its genome does not encode components of the apoptotic pathway. Genetic analysis has allowed distinguishing autophagic from necrotic cell death, and identifying regulatory genes necessary for the first form of death (73, 74).

It is also worth mentioning that the actomyosin cytoskeleton of *Dictyostelium*, since the purification of actin (75) and myosin (76), has been systematically characterized at biochemical, structural, and genetic level (for an early review see (77)). Some actin-binding proteins were found first in *Dictyostelium*, such as coronin (78, 79), the actin nucleator Scar (80), and the 34-kDa actin-cross-linking protein (81). *Dictyostelium* myosin I and myosin II were among the first non-muscle myosins to be characterized (76, 82), and myosin II was one of the first eukaryotic genes to be inactivated by homologous recombination (83, 84), with the surprising result that it was not essential for migration. Structural and functional studies with cytoskeletal components have uninterruptedly continued till our days, as shown by studies with myosin motors (85–87), formins (88, 89), more recently with WASH (90), or studies on the dynamic structure of the cell cortex (91) and on actin cytoskeleton–nuclear membrane interactions (92). The microtubule cytoskeleton

lagged behind the actin cytoskeleton, but in the last 15 years it has gained ground and its role in vesicle trafficking, mitosis, motility, and chemotaxis has been investigated (71, 93–96).

A close look at the inventory of the major biological processes modeled in *Dictyostelium* shows up that most of them are related to phenomena at cellular rather than tissue or organismal level, with the exception in part of pattern formation and social evolution. This is a peculiarity of *Dictyostelium*, when compared to the other nonmammalian model organisms. This also implies that *Dictyostelium* as model is first of all representative, roughly speaking and from an experimental point of view, of different mammalian cell types, be they neutrophils (for motility and chemotaxis) or macrophages (for phagocytosis, macropinocytosis, autophagy, or endo-lysosomal traffic), fibroblasts, epithelial, or other tissue cells (for cell–cell and cell–substratum adhesion), all cell types that are cultured and imaged almost as easily as *Dictyostelium*. Admittedly *Dictyostelium* cells are handled more easily and are less expensive than mammalian cell cultures, but the above-mentioned biological processes can be easily studied in mammalian cells. If we also consider the huge technical tools that are commercially available, in terms of antibodies, molecular biology kits, and probes, all tailored to the needs of mammalian cell research, and mostly of little use for *Dictyostelium* cells, one wonders what makes *Dictyostelium* a competitive model organism in comparison with mammalian tissue cultures. Two advantages can be envisaged, the first one being that *Dictyostelium* integrates at the level of a single cell (genetically much more stable and less artificial than mammalian cell cultures), a variety of biological processes and activities typical of a multicellular organism. This allows for higher reproducibility of experimental results and easier integration of the regulatory networks underlying the biological processes in question, e.g., between chemotaxis and phagocytosis, or phagocytosis, macropinocytosis, and autophagy. The second trump card is genetics, and the ease in combining genetic approaches with cell biological and biochemical ones in the same cell. As long as the genetic tractability of *Dictyostelium* is not equaled by the technological efforts to manipulate genetically mammalian cells, *Dictyostelium* will remain as model in the forefront, like yeast.

4 *Dictyostelium* as Nonmammalian Model for Biomedical Research

As mentioned earlier, the use of nonmammalian animal models for biomedical research stems from the belief that, despite the evolutionary distance and the anatomical, physiological, and developmental differences, information obtained with these organisms is relevant for human pathophysiology. Even if the results from simple

organisms are often not directly translatable to complex organisms, they can offer insights and open avenues that help investigations in mammals and humans. Textbook examples are studies on yeast and sea urchin cell division that led to the identification of genes regulating mammalian cell cycle, and are thus relevant for cancer therapeutics, or research in *C. elegans* death genes that led to the discovery of their orthologs in human cells, fostering research in mammalian apoptosis. On the other side of the coin, it is becoming increasingly evident that even the most used animals in biomedical research, namely, mice and rats, fail often in mimicking human physiology or human diseases (97, 98). Most drug failures in clinical trials, after severe scrutiny in preclinical studies with animals, are related to intrinsic problems linked to differences in the physiology and anatomy of different animal species, apart from methodological shortcomings in animal research standards. The “TGN1412 catastrophe,” namely, the unexpected cytokine release syndrome in a first human trial of a humanized antibody, after negative toxicological tests with rodents and primates (99), is very instructive in this regard. Studies following this fiasco showed years later that rodents were protected against the cytokine storm by a very efficient activation of T regulatory cells, and the monkey species that was used in the preclinical studies failed to express the antibody target in that specific cell type that in humans caused the pro-inflammatory response, a highly unpredictable but crucial difference (100, 101). This and other failures have led to the sober conclusion that animals are better used for understanding disease mechanisms and potential new treatments rather than predicting what will happen in humans (102–104). If so, then in a benefit-cost assessment non-mammalian animal models are a valid alternative to mammals for understanding the mechanism of action of disease genes and of drug treatments. Indeed, nonmammalian model organisms are providing invaluable understanding of the pathophysiology associated with several human diseases (e.g., (105–108)).

With this in mind, what can be the specific contribution of *Dictyostelium* as nonmammalian model for biomedical research? For the reasons mentioned in the previous section, *Dictyostelium* is best used, similarly to yeast, to unravel the mechanism of action of disease genes at cell biological and biochemical level, exploiting the genetic potential of the organism. In this context, *Dictyostelium* multicellular development, although very different from animal development but absent in yeast, is a very important heuristic tool to rapidly detect potential phenotypic effects of disease genes or drugs, which can provide clues on the cellular function and the biochemical mechanism of action of the gene product or the drug under study.

One of the first papers, perhaps the first, advertising *Dictyostelium* as model for human disease genes dates back to 1999 (109). In that paper, Saxe advocated the use of the genetic potential of *Dictyostelium* to investigate questions related to the mechanism

Table 2**Human diseases and biomedical research studies modeled in *Dictyostelium***

Human diseases (<i>Dictyostelium</i> genes in italics) or biomedical research	Most recent reviews, or research papers
Microbial infections and host–pathogen interactions	(115, 138, 139, 149)
Endo-lysosomal traffic diseases (<i>lysB</i> , <i>AP3-μ subunit</i> , <i>mlp1</i>)	(130)
Mitochondrial diseases (<i>Oxphos genes</i> , <i>midA</i> , <i>AMPK</i>)	(123)
Lissencephaly (<i>Ddlis1</i>)	(124)
Huntington disease (<i>hd</i>)	(116, 120, 121)
Shwachman-Diamond syndrome (<i>SBDS</i>)	(116)
Pharmacogenetic studies (drugs: bisphosphonates, cisplatin, lithium, valproic acid)	(136, 137)

of action of Scar/WAVE, the actin nucleator protein first identified in *Dictyostelium* and shortly later in mammals (80). Since then, data have accumulated on other human disease genes playing specific roles in *Dictyostelium* biology. In most cases they were unexpected by-products of studies directly related with *Dictyostelium* biology, such as the above-mentioned Scar (80); the phosphatase and tumor-suppressor protein PTEN, which was defective in a mutant that failed to polarize and chemotax properly (47); the lissencephaly protein Lis1, found to be a component of the centrosome (110); and the *lysB* gene identified in a cytokinesis mutant and found to encode a protein with similarities to the Chediak-Higashi syndrome proteins (111, 112). The proto-oncogene Ras proteins were initially studied as possible regulators of cell proliferation, but it was soon discovered that they were not essential for growth. They were also expressed during development, and were later shown to be important regulators of chemotaxis and phagocytosis (45, 113).

Awareness that *Dictyostelium* could be used for studying disease genes was strengthened by the observation that the *D. discoideum* genome harbors by the lowest estimate 33 homologs of human disease genes (13). In the meantime a few such genes have been studied, and *Dictyostelium* contribution as genetic model for human diseases has been highlighted in a collection of excellent reviews that was recently edited by Ricardo Escalante (114). They cover genes involved in actin cytoskeleton-related diseases, endo-lysosomal traffic diseases (Chediak-Higashi syndrome, Hermansky-Pudlak syndrome, ceroid lipofuscinosis, mucopolipidosis), lissencephaly, mitochondrial diseases, microbial infections, and pharmacogenetics studies (Table 2). An independent review on host–pathogen interactions has also been published (115).

Since these reviews are very comprehensive and very recent, the reader is referred to them for an appraisal of the state of art in each research field. In this context, I will mention two recent contributions that were not covered in the series edited by Escalante, because they illustrate extremely well the power of *Dictyostelium* as nonmammalian model for human disease genes.

4.1 SBDS and the Shwachman-Diamond Syndrome

The functional defect causing the Shwachman-Diamond (SD) syndrome has been recently modeled in *Dictyostelium* by Wong et al. (116). The SD syndrome is caused by recessive mutations in the SBDS gene, resulting in inefficient hematopoiesis, exocrine pancreatic insufficiency, and skeletal abnormalities. The SBDS gene has been implicated in diverse functions, including also 80S ribosome maturation. Premature association of 40S and 60S ribosomal subunits to form the mature 80S ribosome is hindered by the eIF6 protein, which binds to the 60S ribosomal subunit in the nucleolus, and acts as anti-association factor by remaining attached to the 60S subunit in the cytosol (117). In *Dictyostelium*, disruption of the eIF6 gene is lethal, and its expression is up-regulated concomitantly with stages of enhanced protein synthesis, in agreement with its regulatory role in ribosome biogenesis and maturation (118). Since the *Dictyostelium* SBDS gene is also essential, Wong and coworkers conditionally inactivated the gene by using for the first time in *Dictyostelium* temperature sensitive, self-splicing inteins. They found that at the nonpermissive temperature ribosomal subunit joining was markedly impaired, and both growth and development were arrested under conditions or at stages requiring massive protein synthesis. They further showed that the SBDS protein, in association with the GTPase EFL1, directly catalyzes eIF6 removal from the 60S subunit, and thus 80S ribosome formation. These data strongly indicate that the SD syndrome is a ribosomopathy.

The work elegantly illustrates the power of *Dictyostelium* for dissecting the functional defect of a gene by integrating genetics with developmental phenotypes as “readouts” and with cell biological and biochemical assays. Translation of the results to human disease was also achieved: first, by showing that the human SBDS gene, but not variants with disease-causing defects, was able to complement the defect in *Dictyostelium*, and second by validating in human lymphoblasts from patients with SD syndrome that reduction in SBDS protein expression inversely correlated with the severity of 80S maturation defect (116).

4.2 Huntingtin and Huntington Disease

Huntington disease is a progressive neurodegenerative disorder characterized by motor, cognitive, and behavioral disturbances. The disease is caused by expansion of a poly-glutamine stretch in the protein huntingtin, a large HEAT repeat protein that is mostly cytosolic, but also found associated to membrane vesicles, nuclei, and microtubules. The poly-glutamine expansion causes a toxic gain-of-function

phenotype, but also disrupts the normal function of the protein, which however remains largely unknown (119). Two different groups independently disrupted the single huntingtin gene homolog *hd* in *Dictyostelium*, resulting in a plethora of pleiotropic defects, including partial defect in chemotaxis, delayed development, reduced cell–cell and cell–substratum adhesion, and altered postaggregative development, pattern formation, and spore differentiation (120, 121). The most impressive phenotype was a strong cellular sensitivity to osmotic stress (121). This pleiotropic phenotype is reminiscent of the pleiotropic effects found in mutated human cells, and although not yet allowing any conclusion on the underlying biological function of huntingtin, establishes that *Dictyostelium* is a good model for further investigating at cellular level whether a common biochemical mechanism is responsible for the multiple phenotypic defects or whether they reflect different functions of huntingtin.

4.3 Endo-lysosomal Traffic Diseases

The huntingtin mutant, together with a null mutant for the *mlp1* gene encoding the Ca-transporter mucolipin1, responsible for mucopolipidosis type IV (122), and a mutant in the ortholog of AAA+ATPase,97/VCP/cdcD, responsible for the protein aggregation disease IBMPFD (Eichinger, personal communication), are the last entries in the catalogue of mutants in homologs of human disease genes (Table 2). Like the huntingtin mutant, the results so far obtained with all these mutants fall short of the true potential of the organism, nicely shown for the SBDS mutant, although some interesting clues have been obtained, such as the crucial role played by signaling pathways regulated by the AMP-activated kinase (AMPK) in mitochondrial diseased cells (123), or the finding that Lis1, responsible in human for lissencephaly, is a component of the centrosome, in addition to interacting with microtubules and with actin (124).

In particular human disease genes involved in endo-lysosomal traffic are conveniently studied in *Dictyostelium*, as the phago- and macropinosome traffic pathway is well characterized in this organism (26, 27, 125, 126). In addition to the *mlp1* mutant modeling mucopolipidosis, two interesting mutants are defective in *lvsB* and in the AP-3 complex. The *lvsB* gene encodes a homolog of the lysosomal traffic regulator LYST, which in humans causes the Chediak-Higashi-syndrome, a severe lysosomal storage disease (111, 112). The AP-3 mutant is defective in a subunit of the AP-3 complex, responsible for the Hermansky-Pudlak syndrome, characterized by abnormal biogenesis of lysosomes and lysosome-related vesicles (127). Both genes regulate in *Dictyostelium* lysosome to post-lysosome maturation, although at different levels (128–130). Although the mechanism of action of these genes remains unknown in *Dictyostelium* as in other systems, these mutants are among the best cellular model candidates for identifying effector proteins and clarifying the biochemical activity of the involved genes.

Defects in endo-lysosomal traffic and lysosome maturation are at the origin of several lysosome storage diseases (131). *Dictyostelium* is a very valuable model for diseases linked to secretory lysosome-related organelles, because the lysosome is not the dead end of the endo-lysosomal traffic, but it matures into a neutral post-lysosomal vesicle that fuses with the membrane, secreting indigestible material. For the same reason, phenotypic defects of lysosomal storage diseases can be hardly modeled in *Dictyostelium* cells. A recent result may lead to some changes in this regard. Recently, Carnell et al. (90) showed that disruption of the actin nucleation-promoting factor WASH strongly inhibited exocytosis of undigested dextran or colloidal gold. This mutant could be very useful for modeling lysosome storage diseases, by generating in its background double or multiple knockouts in genes encoding lysosomal membrane proteins or hydrolases, such as cathepsins, presenilin, glycosidases, or lipases, for which no or very mild phenotypes exist so far in *Dictyostelium* (130). Needless to say, these cell lines could also be very useful for screening drugs against lysosomal storage diseases.

4.4 Pharma- cogenetic Studies

The potential of the organism for drug screening and for pharmacogenetic studies has been illustrated by studies with aminobisphosphonates, cisplatin, lithium, and valproic acid.

Aminobisphosphonates inhibit osteoclast growth, and are thus used as therapeutics in bone resorption disorders and bone tumors (132). Studies in *Dictyostelium* and human cell lines showed that they were metabolized into non-hydrolyzable ATP analogues (133), and identified farnesyl diphosphate synthase as one of their target of action. Farnesyl diphosphate synthase overexpression was shown to confer resistance to bisphosphonate (134, 135). Pharmacogenetic studies on cisplatin resistance and on mood-altering drugs (lithium and valproic acid) started both with unbiased selection of resistant mutants from a REMI library, and led to the identification of sphingosine-1-phosphate lyase and ceramide as regulators of cisplatin sensitivity in the first case (136), and of the prolyl oligopeptidase and its regulation of phosphoinositide signaling as target of mood-altering drugs in the second case (137), results that in both cases could be confirmed with human cells. The important message from these studies is that *Dictyostelium* is well suited for pharmacogenetic studies, as it allows rapid identification of novel genes targeted by drugs and characterization of their mechanism of action.

4.5 The Macrophage Elder Brother in Host–Pathogen Interactions

An issue that is being vigorously investigated is that of microbial infections and host–pathogen interactions. In a decade of studies *Dictyostelium* has been firmly established as model host for an increasing number of clinically relevant bacterial pathogens (115, 138, 139), the last entry being *Burkholderia pseudomallei*, the causative agent of melioidosis, characterized by acute septicemia and pneumonia (140).

The rationale for these studies is closely linked with *Dictyostelium* biology: as a soil amoeba and professional phagocyte that hunts for bacteria as the obligate source of food, *Dictyostelium* can be a natural host of pathogenic bacteria. Thus its strategies to counteract infection are likely to be of biomedical importance. In addition, many pathogens that only occasionally infect humans are hosted for most of their time in protozoa and free-living amoebae, where selection of their virulence traits occur (141).

Like macrophages, *Dictyostelium* cells have been used for in vivo imaging of the dynamics of infection, exploiting the large collection of cytoskeletal or intracellular traffic proteins fused to fluorescent reporters. These studies have shown that the dynamics of infection is highly conserved between *Dictyostelium* and macrophages (142–147), and in the case of *Mycobacteria* have revealed a novel non-lytic spreading mechanism that may be active also in mammals (148).

Bacterial uptake and proliferation have been investigated in classical phagocytosis and infection assays, exploiting the large number of available knockout mutants to identify genes that confer resistance to a given pathogen (115, 149). Unbiased mutational screen has been also carried out to identify novel host cell factors (150). Genome-wide transcriptional changes during infection and proteomic analysis of pathogen-containing vacuoles have also helped in characterizing the infection process (151–154).

This variety of approaches has produced a large wealth of data, of which only a few will be mentioned here. The use of *Dictyostelium* as test-bed for identifying bacterial virulence factors has led to the characterization of virulence factors in *Vibrio cholerae* (155, 156), *Pseudomonas aeruginosa* (138), and *Legionella pneumophila* (157). Similarly, in *Burkholderia pseudomallei* and *Burkholderia cenocepacia* resistance factors against predation by both *Dictyostelium* and macrophages have been singled out (140, 158).

From the side of the host, infection assays with defined *Dictyostelium* knockout mutants or with a collection of REMI mutants have allowed to discriminate between host “resistance” and host “susceptibility” factors, whose genetic disruption leads to enhanced or reduced pathogen proliferation, respectively. Most of these genes have been identified in infection studies with *Legionella pneumophila* (115). It appears from these studies that *Legionella* needs structurally functional actin cytoskeleton, endoplasmic reticulum, mitochondria, and endo-lysosomal traffic machinery for growth. In contrast, regulatory factors of endo-lysosomal vesicle traffic and fusion, of iron transport in endo-lysosomal vesicles, and of the stress response hinder *Legionella* growth and, if not already compromised as in the knockout mutants, are possibly tagged by *Legionella* (115, 149).

In summary, *Dictyostelium* has proven to be a powerful model for investigating host–pathogen interactions and microbial

infections. The easy generation and analysis of mutants will strengthen its role as a model system complementary to mammalian macrophages, particularly for dissecting the host response and for integrating this response with the pathogen-induced changes in the replicative vacuole.

5 Conclusions

Dictyostelium discoideum is a powerful nonmammalian genetic model system for investigating a large and ever increasing variety of biological processes, from well-established ones, such as actin cytoskeleton-based cell motility, chemotaxis, phagocytosis and endo-lysosomal traffic, collective cell migration during development, and pattern formation, to relatively new roles on stage, such as autophagy and social evolution. It has proven to be an ideal genetic model for host–pathogen interactions and microbial infections, but also for investigating the biological function of several disease genes as well as the mechanism of action of drugs of clinical relevance.

About 25 million mammals, mostly rodents, but also dogs, cats, and primates, are sacrificed each year in the USA and Europe for biomedical research, and yet more than half of the drugs that get through preclinical animal tests fail in clinical trials with human patients. Although medical progress has depended and will depend even in future on animal-based research, alternative approaches may help in implementing the so-called three R policy, namely, refinement of design, carrying out and analysis of animal experimentation, reduction of experiments with mammals whenever possible, and replacement with other techniques. Nonmammalian animal models, including *Dictyostelium*, are a valid and cheaper alternative to reach these objectives, in that they are better suited than mammals for large scale and straightforward genetic analysis of the basic mechanisms of (patho)physiology and of drug action, thus representing a first filter for a more limited and better designed use of mammals.

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