The past, present and future of *Dictyostelium* as a model system

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ABSTRACT The social amoeba *Dictyostelium discoideum* has been a preferred model organism during the last 50 years, particularly for the study of cell motility and chemotaxis, phagocytosis and macropinocytosis, intercellular adhesion, pattern formation, caspase-independent cell death and more recently autophagy and social evolution. Being a soil amoeba and professional phagocyte, thus exposed to a variety of potential pathogens, *D. discoideum* has also proven to be a powerful genetic and cellular model for investigating host-pathogen interactions and microbial infections. The finding that the *Dictyostelium* genome harbours several homologs of human genes responsible for a variety of diseases has stimulated their analysis, providing new insights into the mechanism of action of the encoded proteins and in some cases into the defect underlying the disease. Recent technological developments have covered the genetic gap between mammals and non-mammalian model organisms, challenging the modelling role of the latter. Is there a future for *Dictyostelium discoideum* as a model organism?

KEY WORDS: *Dictyostelium*, chemotaxis, phagocytosis, host-pathogen interaction, cell adhesion

Introduction

The selective use of organisms other than humans has been very critical in biology and medicine for centuries, mostly due to practical reasons that made convenient to study complex biological phenomena in simple species. The emergence of some of these species as “model organisms” has been a recent development, which took place concomitantly with advancement in genome sequencing, leading to a sort of official recognition by the NIH. To quote from the NIH internet site: “Model organisms are a small group of research organisms that serve as a proxy for understanding the biology of humans.. Many aspects of these organisms' biology are similar to ours, and much is already known about their genetic makeup. For these and other reasons, studying model organisms helps scientists learn more about human health”.

As evident from this statement, the model organisms, whatever their evolutionary distance from humans, are functional to the understanding of human biology, leading eventually to knowledge that is relevant for human health. Obviously any organism, which has been selected over the years as object of investigation by a scientific tribe, is an intra-species or intra-genus organism of reference, such as *Saccharomyces cerevisiae* for yeasts, *Drosophila melanogaster* for insects, *Arabidopsis Thaliana* for plants or *Dictyostelium discoideum* for the amoebozoa, but these organisms have reached the model status because they were amenable, more than other organisms, to genetic and molecular analysis of some processes that are relevant for all organisms, including humans. For that reason, if the concept of model organism is clear, the life of a model organism, its emergence, establishment or passing away is fluid and can be favoured or endangered by many factors, such as new technological developments, new biological questions or their re-shaping, and last but not least the emergence of new unforeseen competitors.

Peculiarities and advantages of *Dictyostelium discoideum* as model organism

Among the non-mammalian model organisms, *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. 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 non-lethal mutants can be isolated...
that are affected in some aspects of development, while growing perfectly well.

Wild type Dictyostelium cells are soil amoebae, living in forest detritus in association with a large variety of bacteria, strictly depending on them for growth (Kessin, 2001). The cells are very efficient phagocytes, and being exposed to potential pathogens, they have developed defence mechanisms that are shared in large part with macrophages. Thus, Dictyostelium has been one of the established and preferred model organisms for studying phagocytosis and host-pathogen interactions (Bozzaro et al., 2008). In recent years, symbiotic forms of interactions with some bacteria strains, which are carried by the cells during development as a sort of rudimentary farming, have also been described (Brock et al., 2011). In contrast to bacteria, no reports exist on Dictyostelium wild type isolates carrying viruses, though it is possible that they might be infected by some giant viruses, as it has been shown for

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**Fig. 1. The life cycle of Dictyostelium discoideum.** The different stages of growth and development on non-nutrient agar plate with approximate timing are shown clockwise. (A) Growth-phase cells expressing actin-GFP (green) engulfing yeast particles (red) in a phagocytic cup or a phagosome (Bar: 0.01 mm). (B,C) During aggregation, chemoattractant cyclic AMP relay leads to cell streaming towards aggregation centers. Chemotactically cells are elongated and adhere strongly to each other by end-to-end and lateral contacts (B: 0.1 mm; C: 0.001 mm). (D) Tight aggregates (mounds) form a tip on top of the mound. Cell differentiation into pre-stalk and pre-spore cells in the mounds leads to preferential pre-stalk cell sorting in the tip, which is the source of cAMP that leads to mound elongation into first finger and slug. The tipped aggregates and the slugs are coated with an extracellular matrix secreted by the cells (Bar: 0.1 mm). (E) The slugs undergo migration toward light sources and regions of lower humidity, shedding behind cells and extracellular matrix (Bar: 0.1 mm). (F-H) Upper light favours culmination, leading to formation first of a mexican hat and then of a culminating slug, with the pre-stalk cells moving inward the culminating fruiting body and differentiating into vacuolized stalk cells, that give rise to the stalk of the mature fruiting body, whereas the pre-spore cells accumulate in the sorus, differentiating into mature spores. In (G) pre-stalk cells expressing beta-galactosidase (blue) are shown in the stalk and the tip of the culminating slug (Bar: 0.1 mm). See also Movie in Supplementary Material for aggregation, and text for additional details.
other amoebae (Colson et al., 2017).

Lab strains able to grow axenically in a mixture of peptone and yeast extract, but also in defined minimal media, were selected already in the ’70s of the last century, and since then they have been very useful for classical and molecular genetic studies. Their ability to grow axenically is linked to deletion of a few genes, particularly the one encoding the putative RasGAP NF1 (Bloomfield et al., 2015), which results in fluid-phase uptake by macropinocytosis, a process that is very inefficient, if not absent, in the parental wild type isolates.

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 substrate. Development is triggered by starvation and results in cells acquiring the ability to gather together into aggregates, by secreting and responding chemotactically to cyclic AMP, and to stably adhere to each other by tissue-like adhesive bonds (Fig. 1 and Supplementary Movie 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 coated by a secreted extracellular matrix (Fig. 1). The slugs migrate over the substratum towards light and along temperature gradients. Like animal embryos, each slug has an embryonic organizer - the anterior tip - that regulates collective behaviour, pattern formation, cell fate as well as final morphogenesis (Chisholm and Firtel, 2004, Kessin, 2001). Roughly 80% of the cells in the culminating slug differentiate into spores, whereas the remaining 20% become stalk cells, i.e. highly vacuolized dead cells that form the stalk of the mature fruiting body (Fig. 1). This complex life cycle highlights the uniqueness and the advantages of Dictyostelium as a model organism:

1. The variety of cellular and developmental processes that can be easily studied in Dictyostelium is manifold, and a wide range of biochemical and cell biological assays have been devised over the years, including assays in cell motility and chemotaxis, macropinocytosis, phagocytosis and host-pathogen interactions, cell-cell and cell-substratum adhesion, resistance to osmotic stress, single cell differentiation, caspase-independent cell death and autophagy (Eichinger and Rivero, 2006, 2013), just to mention a few.

2. Essential for a model organism, Dictyostelium cells exhibit rapid growth (3 to 8 hours duplication time, when cultured on bacteria or in axenic culture media, respectively), rapid development (24 hours), small size of mature organisms (micrometer range), making possible to work with statistically high number of cells and organisms. Large yields of cells with defined identity can be easily cultured, facilitating biochemical studies. Growth and development occur optimally at temperatures between 20 and 23°C, under atmospheric CO2 levels, development can be induced by just washing the growing cells in a simple salt solution, and 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.

3. Dictyostelium cells were among the first eukaryotic cells in which in vivo imaging of fluorescent protein chimeras was applied, both at the level of single cells and three-dimensional organism (Gerisch et al., 1995, Müller-Taubenberger, 2006), 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, though 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 manipulate genes (Eichinger and Rivero, 2006). Until recently, only axenic strains were amenable to molecular genetic treatments, but protocols are now available to efficiently transfect and manipulate wild type strains growing on bacteria (Paschke et al., 2018).

Dictyostelium is haploid, therefore gene disruption by homologous recombination usually causes phenotypes without the need for further manipulation. Multiple knockout mutants can be created by Cre/LoxP-mediated recombination (Faix et al., 2004, Linkner et al., 2012), thus facilitating analysis of gene families or gene networks. Recombination or para-sexual complementation is possible only with a few selected strains (Bloomfield et al., 2019), but mutants may be generally rescued by introducing the gene of interest in wild type or mutated form. In addition to its use for disrupting genes by homologous recombination, restriction enzyme mediated integration (REMI) has been used for suppression genetics (Shaunsly et al., 1996).

Recently, a Genome Wide Dictyostelium Insertion (GWDI) project based on REMI-seq technology has led to the establishment of a collection of 5.705 single knockout mutants, covering 42% of all annotated genes (https://remi-seq.org/). Libraries and handling protocols for analysing these mutants are available in the Dicy stock center (www.dictybase.org), which also offers a wide selection of plasmids, mutants and stable cell lines with ablated or overexpressed genes. A cDNA library representing 55% of all genes expressed at different developmental stages is also available (Urushihara et al., 2006). Novel genetic tools, such as Crispr/ Cas9-mediated genome editing, have been recently shown to be applicable to Dictyostelium cells (Muramoto et al., 2019).

4. Critical for successful model organism research is the establishment and maintenance of infrastructures enabling crossdisciplinary communication and exchange of materials, including stock/strain centers and databases for rapid communication of research. As just mentioned, a centralized Dicty stock/strain center was established already 30 years ago, together with a community resource database (www.dictybase.org), where curated and annotated gene models as well as standardized research techniques, a comprehensive reference library and several genetic tools can be browsed.

Past and present of a model organism

As mentioned in the introduction, a lower organism has been considered valuable as model in so far as it is possible to study at molecular and genetic level, thus “modeling”, a relevant biological process otherwise difficult to tackle in mammals or humans. The identification of homologous, and even more, orthologous genes products with similar biochemical and molecular function between a lower model organism and mammals, has strengthened this role. We will see in the last section that recent technological developments are challenging the role of lower model organisms. However, before dealing with this question, it is worth mentioning what has been the contribution of Dictyostelium as a model organism, and which processes have been successfully modelled so far in this organism. This was the subject of a detailed review published a few years ago (Bozzaro, 2013), which the reader is referred to. In this short report I will only summarize the major topics and mention some relevant papers or reviews that have been published in the
last 6 years after that review (Table 1).

*Dictyostelium* has been and still is a leading model for eukaryotic chemotaxis (Devreotes and Horwitz, 2015), and one of the established models for other motility-linked processes, such as cytokinesis (Srivastava et al., 2016), phagocytosis (Bozzaro et al., 2008), macropinocytosis and endo-lysosomal traffic (Williams and Kay, 2018); (Williams et al., 2019). Concerning cell motility, an uninterrupted series of studies in the last 50 years has led to the identification and characterization of the acto-myosin cytoskeleton underlying changes in cell shape and cell motility processes, with many cytoskeletal proteins first identified and/or characterized in *Dictyostelium*, such as coronin, the actin nucleator SCAR, the 34-kDa actin-crosslinking protein, myosin I and II, and formins (see Bozzaro, 2013 for references). The dynamic structure of the cell cortex, the actin cytoskeleton-nuclear membrane interactions, nucleus/nucleolus and the microtubule cytoskeleton, and their regulation by small GTPases have been the subject of several studies also in recent years (Nichols et al., 2015; Graf et al., 2015; Rivero and Xiong, 2016; Meyer et al., 2017; Pitzen et al., 2018). Recently, the role of formins in regulating the functional integrity of the cell cortex has been investigated in detail (Junemann et al., 2016), and it has been shown that the HSBP1 protein, which regulates WASH complex assembly at centrosomes, is required for development of focal adhesion and cell polarity in *Dictyostelium* as well as in tumour cells (Visweshwaran et al., 2018). Arginylation, a post-translational modification regulated by the conserved enzyme arginyl-tRNA-protein (Ate1), has been shown to control actin polymerization, affecting focal adhesion and motility (Batsios et al., 2019). Evidence has also been provided that microtubules control mitochondria fission, fusion and motility (Woods et al., 2016).

The dynamics of chemotaxis and its regulatory pathways have continued to be the subject of intense studies in *Dictyostelium*, in particular for understanding how the different signalling networks are wired together, and for generating models of random and oriented cell motility in 2- and 3-D, often anticipating results and insights that have promoted research in neutrophils and other organisms (Stuelten et al., 2018; van Haastert et al., 2018; Liu et al., 2018; Li et al., 2018; Nichols et al., 2015; Edwards et al., 2018).

Among recent contributions, it is worth mentioning that chemotactic signal relay has been shown to be mediated by released exosomes, containing adenylyl cyclase and cAMP, the latter being secreted via an ABC transporter (Kriebel et al., 2018). Extracellular vesicles as a form of detoxifying mechanism had been already described in *Dictyostelium* long ago (Tatischeff, 1998), and this latter result confirms that they can act as potential carrier of biological information.

A phospho-proteomic approach has led to the identification of the atypical ERK2 kinase as a sort of master regulator of the signalling network that drives chemotaxis (Nichols et al., 2019). ERK2 knockout abolishes folate and cAMP chemotaxis, confirming its essential role in controlling chemotaxis (Schwebs et al., 2018).

Sensitivity to chemotactic signals appears to be negatively and positively controlled by PKA, HECT ubiquitin ligase-dependent ubiquitination and a novel negative regulator of Ras signalling (Scavello et al., 2017; Pergolizzi et al., 2017; Xu et al., 2017), whereas stochastic as well as chemotactically oriented pseudopod formation could be driven by a coupled excitable Ras/PI(3-4)P2/F actin system (van Haastert et al., 2017; Li et al., 2018).

A structural study of the G protein interacting protein 1 (Gip1) has revealed how Gip1 binds and sequesters the heterotrimeric G protein, regulating G protein-coupled receptor signalling in chemotaxis (Miyagawa et al., 2018). The same group has also elegantly shown that the heterotrimeric G protein dynamics is able to adapt to different concentrations of cAMP, explaining chemotaxis over a wider concentration range of the chemoattractant (Miyagawa et al., 2018).

Research with *Dictyostelium* cells was very influential in the '70-'80 of the last century for establishing that intercellular adhesion was mediated by specific membrane proteins, against the at that time prevailing view that cell adhesion simply depended on the sum of attractive and repulsive forces operating at the cell surface, irrespective of specific “adhesion molecules”, whose search for was considered irrelevant. The successful immunological strategy developed with *Dictyostelium* cells for the identification of the contact sites A and B (csA and csB) (Beug et al., 1973; Gerisch, 1980), was soon adopted in mammals, leading to the identification of N-CAM (Brackenbury et al., 1977) and the first cadherin (uvomorulin, later called cadherin E) (Hyafil et al., 1980). The finding a few years later that disruption of the gene encoding the homophilic adhesion glycoprotein csA, though being essential for EDTA-stable adhesion during the aggregation stage, failed to affect development (Noegel et al., 1985), represented a temporary drawback, overcome when it was shown that the apparent redundancy was due to the standard laboratory conditions not being as stringent as those in the natural environment (Ponte et al., 1998). This led indirectly to the csA encoding gene being recognized as the first “greenbeard” gene, promoting research of greenbeard genes involved in kin discrimination in other organisms (Queller et al., 2003; Gardner and West, 2010; Strassmann, 2016; Heller et al., 2016).

### TABLE 1

<table>
<thead>
<tr>
<th>Biological process</th>
<th>Most recent reviews or relevant papers</th>
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<tr>
<td>Cytoskeleton, motility and chemotaxis</td>
<td>Graf et al., 2015; Rivero and Xiong, 2016; Stuelten et al., 2018; Liu et al., 2018; van Haastert et al., 2018; Edwards et al., 2018; Kriebel et al., 2018; Nichols et al., 2015; Miyagawa et al., 2015</td>
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<td>Phagocytosis, macropinocytosis and endo-lysosomal traffic</td>
<td>Williams and Kay, 2018; Williams et al., 2019; Pan et al., 2018; Meena and Kimmel, 2017; Buckley et al., 2019; Dunn et al., 2018; Dinh et al., 2018; Marinovic, 2019</td>
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<td>Cytokinesis</td>
<td>Srivastava et al., 2016</td>
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<td>Cell adhesion</td>
<td>Fujimori et al., 2019; Lampert et al., 2017</td>
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<td>Autophagy</td>
<td>Mesquita et al., 2017; Fischer et al., 2019</td>
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<tr>
<td>Development, pattern formation</td>
<td>Yamada and Schapa, 2019</td>
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<tr>
<td>Social evolution</td>
<td>Ostrowski, 2019</td>
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Only selected reviews and/or papers published after 2013 are listed. For additional references see text.
The immunological approach led also to identification of a 150 kDa glycoprotein, encoded by the lagC1 gene, mediating heterophilic adhesion at the postaggregative stage (Geltosky et al., 1979). Later it was shown that the LagC1 and LagB1 glycoproteins (now called TgrB1 and TgrC1) mediated heterophilic adhesion and were responsible for differential adhesiveness between prespore and prestalk cells at the slug stage (Benabentos et al., 2009; Hirose et al., 2017). Recently, it has been elegantly shown that this form of adhesion cooperates, in a cell-type specific manner, with cAMP-driven chemotaxis in mediating 3-D cell migration and cell sorting, thus pattern formation, during slug morphogenesis (Fujimori et al., 2019). It is likely that this result will have an impact in mammalian cell sorting out studies.

Dictyostelium cells are very suited to biophysical studies of cell-cell and cell-substratum adhesion, as shown by several reports in the past and in recent years (Kamprad et al., 2018; Lampert et al., 2017; Zhu et al., 2015; Tarantola et al., 2014; Wang et al., 2014; Schindl et al., 1995; Helenius et al., 2018; Heinrich et al., 2015). Although it was well known that Dictyostelium cells grow by ingesting bacteria, systematic studies on the molecular basis of phagocytosis and macropinocytosis started relatively late, namely in the '90s of the last century, almost concomitantly with investigations on host-pathogen interactions. Since then, these lines of research have been vigorously pursued by several labs, firmly establishing Dictyostelium as a model for professional phagocytosis, macropinocytosis and as model host for an increasing number of clinically relevant bacterial pathogens. Dictyostelium cells have been used for in vivo imaging of the dynamics of phagocytosis, macropinocytosis and infection, exploiting the large collection of cytoskeletal or intracellular traffic proteins fused to fluorescent reporters. Genetic studies involving knockout mutants, unbiased mutational screens, genome-wide transcriptional changes and proteomic analysis during phagocytosis, macropinocytosis or infection have produced a large wealth of data, which have been the subject of several reviews in the last years (Bozzaro and Eichinger, 2011; Dunn et al., 2018; Cardenal-Muñoz et al., 2018; Swart et al., 2018). Among more recent data not yet covered by reviews, it is worth mentioning: (I) evidence that Dictyostelium cells are highly sensitive to bacterial chemotactants, but phagocytosis per se is independent of chemotaxis (Meena and Kimmel, 2017); (II) in apparent contrast to this, the characterization of the G protein-coupled, folic acid receptor fAR1, which recognizes the saccharide core of LPS and stimulates gram-negative bacterial phagocytosis, providing a plausible mechanism for the involvement of G proteins in phagocytosis (Pan et al., 2018); discrepancy between both results may be due to the different phagocytosis assays used; (III) evidence that Dictyostelium discriminates between Gram-(-) and Gram-(+) bacteria, migrating preferentially toward Gram-(-) bacteria (Rashidi and Ostrowski, 2019), and that their growth on Gram-(-) or Gram-(+) bacteria elicit different transcriptomic profiles, with some genes essential for growth (Nasser et al., 2018); (IV) the identification of PIKFyve/FAB1 in controlling acidification of phagosomes (Buckley et al., 2019), thus extending previous studies on the role of phos- phoinositides in phagocytosis, macropinocytosis and resistance to pathogens (Swart et al., 2018; Hoeller et al., 2013; Percacio et al., 2010; V) Similarly, it has been shown that the RasGAP IgGc is a negative regulator of macropinocytosis and large particle phagocytosis (Marinovic et al., 2019), strengthening the role of Ras signalling in these processes (Williams et al., 2019; Junemann et al., 2016; Bolourani et al., 2010; VI) While bacterial phagocytosis is essential for growth, it has also been shown that during slug stage, cells maintain as endosymbionts bacteria that have been coated with the secreted lectin discoidin I, and that could possibly be used later as a food source (Dinh et al., 2018).

Concerning host-pathogen interactions, zinc poisoning, in addition to iron restriction, has been further investigated in Dictyostelium interactions with Legionella pneumophila, Mycobacterium marinum or Escherichia coli (Buracco et al., 2018; Barisch et al., 2018), whereas it has been definitely shown that the orthologous iron transporter Nram1 induces iron efflux from the phagosome (Buracco et al., 2015), and acts as resistance factor, in addition to Legionella and mycobacteria, also against Francisella infection (Brenz et al., 2017). Evidence has also been provided that the ESCRT machinery and autophagy cooperate in controlling mycobacteria infection (López-Jiménez et al., 2018), and that saposin-like proteins, which are encoded by several genes in Dictyostelium, do have amoeboapore-like activity (Dhakhshunamoorthy et al., 2018).

Detailed investigations with Legionella pneumophila during the last 15 years have shown that intracellular growth of the pathogen needs structurally functional actin cytoskeleton, endoplasmic reticulum, mitochondria and the endo-lysosomal traffic machinery. 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 (Bozzaro and Eichinger, 2011; Steiner et al., 2018; Steiner, 2011). Similarly to Legionella, regulation of phosphoinositide pattern in the replicating vacuole by phosphatases controls replication of Mycobacterium marinum in Dictyostelium and macrophages (Koliwer-Brandi et al., 2019). In addition to Legionella and mycobacteria, which have been much studied in their interactions with Dictyostelium, investigations with Salmonella enterica serovar Typhimurium and Klebsiella pneumoniae have led to the identification of different traits as virulence factors (Varas et al., 2018); (Marcotea et al., 2018). 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.

Studies on autophagy and apoptosis-independent cell death started with a pioneering paper in 2003 (Otto et al., 2003), and in the last 15 years have shed light on the mechanisms that regulate autophagosome formation (Mesquita et al., 2017), and on the involvement of autophagy in defence against bacterial infections (López-Jiménez et al., 2018), in development and cell differentiation (Fischer et al., 2019; Yamada and Schaap, 2019). Dictyostelium has also emerged in the last years as a powerful simple model for genetic analysis of social evolution (Ostrowski, 2019), a topic that is covered in this issue by the review of J. Strasman, which the reader is referred to.

**Dictyostelium as a model for biomedical research: recent contributions**

The occurrence in the Dictyostelium genome of several genes homologous to disease genes in humans has stimulated the use
of Dictyostelium as model for dissecting the biological function of these genes and the mechanism of action of the encoded proteins. Dictyostelium cells have also been very useful as test-bed for pharmacogenetic studies. In the previous review (Bozzaro, 2013) and in a collection of dedicated papers (Escalante, 2011), these topics were covered in detail (see also Table 2), thus I will only mention major publications published in the last six years.

The finding that mitochondrial respiratory deficiencies induce in Dictyostelium a consistent pattern of altered phenotypes has been further exploited to investigate genes involved in mitochondrial diseases (Annesley et al., 2014). It has been shown that mutations in the Dictyostelium homologues of two Parkinson’s disease associated proteins, DJ-1 and HTRA2, have differential effects on mitochondrial dysfunction (Chen et al., 2018; Chen et al., 2017).

Several papers have studied genes whose variants are involved in the neuronal ceroid lipofuscinosis (NCL) Batten disease. Knockdown or knockout of proteins, such as TPP1 (Tripeptidyl peptidase), Cln3 or Cln5, affected Dictyostelium cell growth and development. Interestingly, Cln3 disruption triggers TPP1 as well as Cln5 up-regulation, providing evidence for molecular networking of NCL proteins (Smith et al., 2019; Huber and Mathavarajah, 2018; McLaren et al., 2019; Phillips and Gomer, 2015; Huber et al., 2014). Cln5 has been also shown to be secreted and to act as glycoside hydrolase both in Dictyostelium and in human cells. Potential interactors have also been identified (Huber and Mathavarajah, 2018). Among these interactors is the Golgi pH regulator (GPHR) protein, which binds to TPP1 via domains that are also present in the mammalian proteins. Whether this interaction is important for the disease is open (Stumpf et al., 2017).

Dictyostelium Roco4 kinase had been successfully used as model to study the structural and biochemical characteristics of the human LRRK2 kinase, whose mutations are the most frequent cause of dominant inherited Parkinson disease (Gilsbach et al., 2012). The same authors have then characterized the structure of two inhibitors bound to mutated Roco4, showing that this system can be used for optimizing LRR2K inhibitors (Gilsbach et al., 2015). Based on these studies, a new model of LRKK2 activation has been recently proposed (Wauters et al., 2019).

It was already known that Dictyostelium cells are quite resistant to protein misfolding and aggregation, a hallmark of several neurodegenerative diseases (Santarriaga et al., 2015; Malinovska et al., 2015). Recent work has allowed to identifying the serine-rich chaperone protein 1 (SRCP1) as a molecular chaperone necessary and sufficient in Dictyostelium to suppress poly-Q expanded protein aggregation, leading to their degradation in proteasomes (Santarriaga et al., 2018).

The potential of Dictyostelium cells for drug screening and pharmacogenetic studies had been illustrated in the past with studies with aminobisphosphonate, cisplatin, valproic acid and lithium (Alexander et al., 2013; Otto et al., 2016). These studies have now been extended to the characterization of curcumin and naringenin, which were found to inhibit development and/or growth (Cocorocchio et al., 2018; Swatson et al., 2017; Garie and Walters, 2015; Waheed et al., 2014).

The polyketide Differentiation Inducing Factors (DIFs) 1-3, which are produced by Dictyostelium cells and induce stalk cell differentiation (Masento et al., 1988), and several of their synthetic derivatives have been shown to have not only anti-tumoral (Arioka et al., 2017; Dubois et al., 2016; Kubokura et al., 2015; Takahashi-Yanaga et al., 2014), but also anti-microbial activities, interestingly against Gram-positive, but not Gram-negative, bacteria (Kubohara et al., 2019), suggesting that Dictyostelium can be an important source in drug discovery (Kubohara and Kikuchi, 2018).

**Is there a future for Dictyostelium as a model organism?**

From this very short survey it is evident that Dictyostelium has been so far a very useful model organism, in particular for unraveling cell biological processes. It is likely that many of the research lines outlined above will be pursued also in the near future.

The utility of non-mammalian model organisms for the understanding of human biology, and ultimately human diseases, however, has been questioned in the last years due to impressive technological progress that has made possible studying mammals to an extent unforeseen a few years ago. In addition to the huge array of biochemical and molecular biology tools, tailored for research with mammalian and human cells, genome-wide association studies and whole genome sequencing has facilitated the direct discovery of disease genes and variants in humans. New genetic tools, such as CRISPR/Cas9, have made mammalian and human cells accessible to genetic manipulation and editing, reducing the genetic gap with lower model organisms. Furthermore, disease modelling can be done with increasing effectiveness using human induced pluripotent stem cells or 3-D organoids (Wang et al., 2019; Chen and Knoepfler 2016; Robbins and Price, 2017; Dutta et al., 2017; Ortiz-Vitali and Darabi, 2019). In view of these developments, and in consideration of the increased competitiveness and pressure for research funding, it is difficult to anticipate what could be the future of Dictyostelium as a model organism.

There are, clearly, some limitations that make studies with lower organisms still unavoidable (Rine, 2014). Human disease phenotypes in many cases do not provide clues about the underlying molecular defect, thus unbiased genetic experiments in experimentally tractable organisms are required to establish

<p>| TABLE 2 |
| Human diseases and biomedical research studies modelled in Dictyostelium |</p>
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<tr>
<th>Human disease or biomedical research</th>
<th>Most recent reviews or relevant papers</th>
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<tr>
<td>Microbial infections and host-pathogen interactions</td>
<td>Cardenal-Muñoz et al., 2018; Swart et al., 2018; Steiner et al. 2018; Kolwier-Brandt et al. 2019; Brenz et al. 2017</td>
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<td>Mitochondrial diseases</td>
<td>Chen et al. 2017, 2018; Annesley et al. 2014</td>
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<td>Neuronal lipid lipofuscinosis</td>
<td>McLaren et al. 2019; Smith et al., 2019</td>
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<tr>
<td>Poly-Q diseases</td>
<td>Santarriaga et al. 2018; Malinovska and Alberti, 2015</td>
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<tr>
<td>Shwachman-Bodian-Diamond syndrome</td>
<td>Weis et al., 2015</td>
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<tr>
<td>Pharmacogenetic research</td>
<td>Wauters et al., 2019; Cocorocchio et al. 2018; Kubohara and Kikuchi, 2018; Kubohara et al. 2019; Arioka et al. 2017; Dubois et al. 2016</td>
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causal mechanisms of gene action (Aitman et al., 2011). Embody-
atically, evidence that the SBDS (Shwachman-Bodian-Diamond
syndrome) protein, whose mutations cause bone marrow failure
and leukaemia in humans, is required for translational activation
of ribosomes, thus that SBDS is a ribosomopathy, was not originated
from observation or analysis of the disease phenotypes, but from
unbiased studies first in yeast and then in Dictyostelium (Menne et
al., 2007; Wong et al., 2011; Weis et al., 2015). This case shows
that lower organisms are better suited than mammals or human cell
lines for large scale and straightforward genetic analysis of patho-
physiological mechanisms, and as such they are an important filter
for an ethically responsible and better designed use of mammals,
thus implementing the so-called 3-Rs policy, namely Refinement
of experimental design, Reduction of experiments with mammals,
Replacement with other techniques.

It is increasingly evident that biochemical and molecular func-
tion of a given protein are generally conserved among different
organisms. The "organismal"-level phenotypes, however, may be
different in different species, even for conserved genes. Thus, PKA
or GSK are kinases in all organisms, but their disruption affects
chemotaxis and development in Dictyostelium, nutrient and stress
signalling in yeast, neuronal plasticity and neurodegenerative dis-
orders, among a plethora of other phenotypes, in mammals. The
emergence of different phenotypes is a consequence of the fact
that the relationships between genes and phenotypes are manifold,
but this obviousness underlines a major role of model organisms.
Besides helping understanding the biological mechanism or function
of a given gene, a model organism is important because it helps
integrating genes in gene networks, and gene networks in biological
contexts i.e. phenotypes, which are species-specific but in some
way equivalent between different model organisms, though their
recognition is not immediate. Unraveling the complexity of biologi-
cal processes behind the simpler phenotypes of lower organisms
eventually helps their translation to mammalian and human biology.
This may lead to a better understanding of human diseases as a
consequence. From this point of view, it is worthwhile to remind a
few aspects that make attractive Dictyostelium:

(1) This organism integrates at the level of the same cell a
large variety of cellular processes that can be used as readouts of
gene functions (during growth: mitosis and cytokinesis, macropi-
nocytosis, phagocytosis, host-pathogen interactions, cell motility
and chemotaxis toward bacteria, cell-substratum adhesion; upon
starvation: chemotaxis driven by cAMP signalling, cell polarization,
cell-cell and cell-substratum adhesion, cell streaming and cellular
oscillations; in the multicellular stage: cell differentiation into a few
cell lineages (pre-spore cells, pre-stalk cells, anterior-like cells,
 sentinel cells) coupled with pattern formation and morphogenesis
(3-D cell migration, phototaxis and cell sorting within the slug and
the culminating fruiting body). Inactivating or overexpressing one
or more genes can affect any one of these "phenotypes", thus fa-
vouring a systematic analysis of genes and their encoded proteins;

(2) Dictyostelium offers the opportunity for a comprehensive
understanding of the biological principles regulating each one of the
processes listed above at the level of a whole organism, which will
still be very difficult in future with mammals. Dictyostelium shares
this opportunity with yeast, but the variety of biological processes
that can be modelled in Dictyostelium is much larger than in yeast.
Most of the 13,000 genes in the Dictyostelium genome are now
annotated with functional information in the Dictyostelium genome
database, and more than 5,000 single mutants with known gene
 disruption are now available. By analysing these mutants and
generating epistatic gene interactions, coupled with "omics" tech-
nologies, Dictyostelium researchers are in a position to unravel
the pathways involved in each one of the processes mentioned

![Dictyostelium fruiting bodies formed on garden soil. Under standard laboratory conditions, Dictyostelium development is studied on non-nutrient agar plates. The smooth, hydrophilic agar surface is highly artificial compared to the forest soil where Dictyostelium cells normally live. It is possible to reproduce in the lab conditions which are closer to the natural ones, by using commercially available garden soil, eventually of different texture (sandy, loamy or clay) and with different degrees of moisture. The surface of any garden soil is rough, much less uniform than agar, with “mountains and valleys” that cells have to overcome for undergoing first aggregation and then slug migration. Under these more stringent conditions it is possible to detect phenotypes of mutants, compared to parental cells, which are otherwise undetectable on agar, thus facilitating the study of the effects on development of prima facie “redundant” genes (see Ponte et al., 1998, 2000).](image-url)
above and how they are wired together.

(3) Experiments in Dictyostelium, as well as other organisms including mammals, are usually conducted under defined laboratory conditions, which are not the environment in which they have evolved. This has led to apparent redundancy in many experimental systems, i.e. disruption of presumably functional genes with no obvious phenotype (Rine, 2014). It has been shown that it is possible for Dictyostelium to reproduce in the lab conditions that are closer to the environmental ones (Fig. 2), thus revealing phenotypes of disrupted genes that were not detectable under standard laboratory conditions (Ponte et al., 2000, 1998). This opportunity should be exploited in future in a more systematic way, provided now the large library of mutants available.

(4) A critical mass of investigators working on the same organism and sharing common tools and resources is required for model organism research to maintain the infrastructures required by the community and to survive in the highly competitive field. The Dictyostelium community is relatively small, compared e.g. to the yeast or Drosophila community. This could be a serious handicap in future, but also an opportunity for young talented scientists that will recognize and exploit the potentialities of what can be considered a "model organism for all seasons", while avoiding overcrowded fields of research.

References


Dictyostelium as model organism


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