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Review

Simple system – substantial share: The use of *Dictyostelium* in cell biology and molecular medicine

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ABSTRACT

Dictyostelium discoideum offers unique advantages for studying fundamental cellular processes, host–pathogen interactions as well as the molecular causes of human diseases. The organism can be easily grown in large amounts and is amenable to diverse biochemical, cell biological and genetic approaches. Throughout their life cycle *Dictyostelium* cells are motile, and thus are perfectly suited to study random and directed cell motility with the underlying changes in signal transduction and the actin cytoskeleton. *Dictyostelium* is also increasingly used for the investigation of human disease genes and the crosstalk between host and pathogen. As a professional phagocyte it can be infected with several human bacterial pathogens and used to study the infection process. The availability of a large number of knock-out mutants renders *Dictyostelium* particularly useful for the elucidation and investigation of host cell factors. A powerful armory of molecular genetic techniques that have been continuously expanded over the years and a well curated genome sequence, which is accessible *via* the online database dictyBase, considerably strengthened *Dictyostelium*'s experimental attractiveness and its value as model organism.

Introduction

Since the advent of cell biological research, scientists have selected easily accessible and powerful model systems to advance their studies. Notwithstanding the progress achieved in the experimental tractability of higher organisms, 'simple model organisms' became extremely valuable tools for investigating fundamental biological questions and also increasingly contributed to clinical research. Their use is beneficial as they help to (1) overcome ethical and experimental limitations; (2) reduce animal death and/or adverse experimentation; (3) optimize and standardize analytical methods; and (4) verify and generalize findings relating to a variety of biological processes.

The available methods and the research objectives influenced the selection and use of model organisms over time. One of the oldest models is the medusozoan *Hydra*, which was described 300 years ago in microscopical studies by van Leeuwenhoek (1702). Today, *Hydra* is used to explore the mechanisms underlying regeneration in the adult organism and to understand the various signaling cascades involved. Other famous historical models are sea urchins and amphibian embryos. With the former Theodor Boveri elucidated more than a hundred years ago the basic principles of cell division, and ontogenetic studies by Hans Spemann with the latter led to the description of the *Spemann organizer* in 1924 (Spemann and Mangold, 1924). A few years later, Thomas Hunt Morgan showed in *Drosophila melanogaster* that each chromosome is made up of a chain of discrete particles which he called genes because they are the physical basis of the genealogy of every individual and thus confirmed earlier evolutionary theories (Morgan, 1927). These few examples illustrate the crucial role of these "old" models to solve fundamental biological questions.

Nowadays, a number of additional "simple" organisms like yeast, *Dictyostelium discoideum*, *Neurospora crassa* or *Caenorhabditis elegans*, to name just a few, are employed to investigate a wide range of cellular processes including, for example, signaling pathways. The resulting knowledge helps us to understand the biology of more complex species including humans that are much more difficult to study directly. For the researcher the work with model organisms provides a number of experimental advantages. These species are generally widely explored, easy to maintain under laboratory conditions and diverse tools for their manipulation and analysis are available. On the other hand there are also shortcomings, and each model organism has its specific advantages and disadvantages. The principle question is whether research using model organisms allows scientists to achieve the ultimate goal: to understand human diseases on a molecular basis and to develop cures. This requirement is extremely hard to fulfill and

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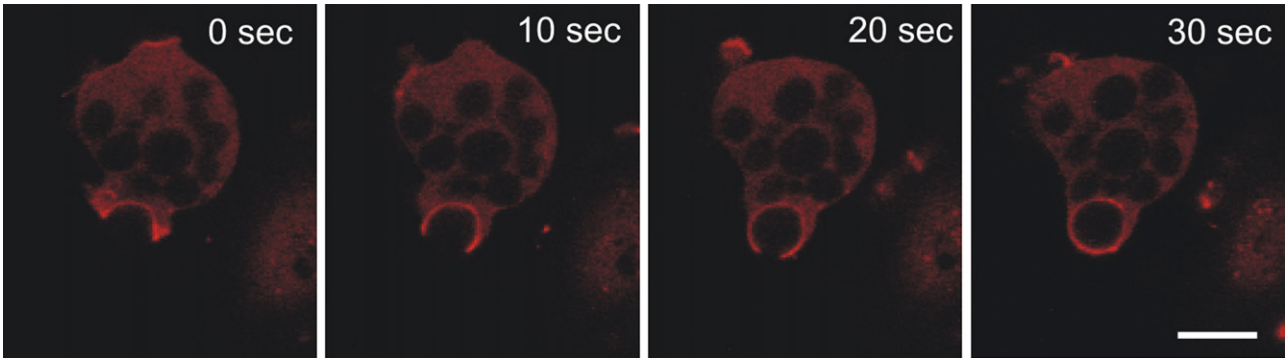

Fig. 1. Phagocytosis of yeast by a *Dictyostelium* cell. Expression of mRFPmars-LimEΔ was used to visualize filamentous actin and to follow the formation of the phagocytic cup. The bar corresponds to 10 μm.

a *universal* model organism does not exist. However, the various model systems allow the study of a range of common biological processes which are crucial for proper cell function and out of balance in the diseased state. Currently, the National Institutes of Health list twelve model organisms for biomedical research (http://www.nih.gov/science/models). The mammalian models are mouse and rat. The rat is, however, much less frequently used in research because its genome is less tolerant with respect to insertion of foreign DNA as compared to the mouse, and this makes it less suitable for genomic manipulations. The non-mammalian models comprise the bird *Gallus gallus*, the fish *Danio rerio*, the amphibian *Xenopus laevis*, the fly *D. melanogaster*, the worm *C. elegans*, the filamentous fungus *N. crassa*, the water flea *Daphnia pulex*, the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, and the amoebozoan *D. discoideum*.

Dictyostelium discoideum – a model used for many reasons

D. discoideum belongs to the social amoebae (*Dictyostelia*). Phylogenetically, *Dictyostelia* form a subbranch in the amoebozoa, one of the six kingdoms into which eukaryotes are currently partitioned. In evolutionary terms they are approximately as distant from yeast as from humans, and they are positioned at the border between uni- and multicellularity. Global protein sequence comparisons, made possible by the genome sequence, showed that *D. discoideum* is evolutionarily closer to animals and fungi than to plants (Eichinger et al., 2005). More recently, the genomes of three other *Dictyostelia*, *Dictyostelium fasciculatum*, *Dictyostelium purpureum* and *Polyspondylium pallidum*, confirmed this (Heidel et al., 2011; Sucgang et al., 2011).

D. discoideum lives in the soil and feeds on bacteria and other microorganisms that are taken up by phagocytosis (Fig. 1). During the vegetative growth stage, the single-celled amoebae divide by simple mitotic divisions. In times of starvation, a developmental program is initiated, which is accompanied by major changes in gene expression (van Driessche et al., 2002). As a result, cells begin to signal each other by secreting cAMP and to aggregate by chemotaxis toward this chemoattractant (Fig. 2A–E). The resulting multicellular aggregate contains up to a few hundred thousand cells and undergoes further differentiation and morphogenetic changes (Fig. 2F and G). Finally a fruiting body is formed (Fig. 2H) which consists of two main cell types, spore and stalk cells. The stalk consists of dead vacuolated cells, while the spore cells are resistant to extreme temperatures or drought. More favorable environmental conditions enable the hatching of new amoebae from the spores. The aggregation of thousands of individual cells that build a multicellular organism in this peculiar life cycle, has intrigued scientists for decades since the first description by Kenneth Raper, and is responsible for the addendum 'social' in the name (Raper, 1935).

Of the many pioneering studies that strengthened the use of *Dictyostelium* as a model, a few examples are highlighted in the following section.

(1) Studies on cell adhesion during early aggregation of *Dictyostelium* led to the identification of a membrane glycoprotein, the contact sites A (csA) protein that mediates specific cell-cell contacts. The strategy and the methods to identify csA were

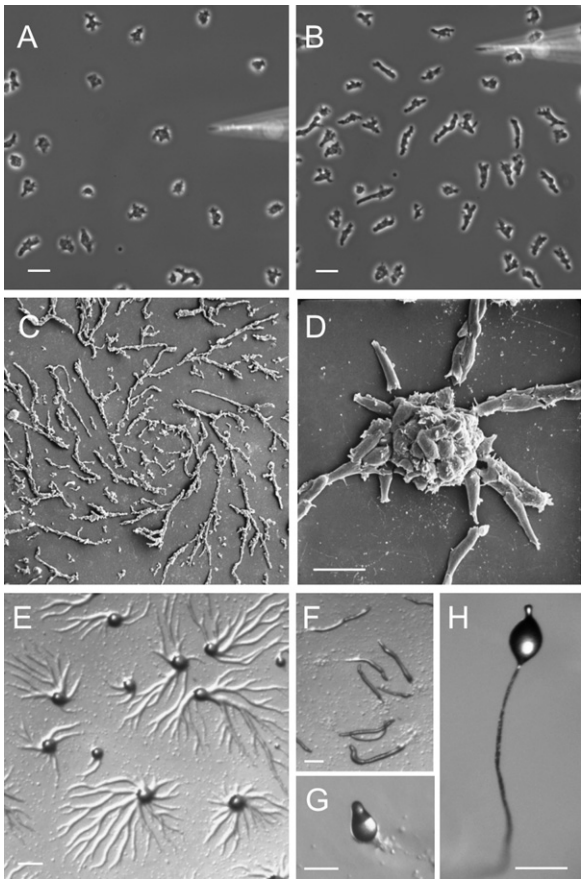

Fig. 2. Stages of the *Dictyostelium* life cycle. (A and B) *Dictyostelium* cells chemotaxing toward cAMP released from a micropipette. Cells that have not yet sensed cAMP are shown in (A). Within 1 or 2 min the cells polarize (note the elongated cell shape) and migrate toward the source of chemoattractant (B). (C and D) Scanning electron micrograph of streaming *Dictyostelium* cells (C) and the formation of aggregates (D). (E) Formation of aggregation centers on an agar plate. (F) Slugs moving on an agar plate. (G) Culmination stage. (H) Fruiting body. Figures (C) and (D), courtesy of Günther Gerisch and Michael Claviez. The bars correspond to 10 μm in (A), (B) and (D), and 1 mm in (E)–(H).

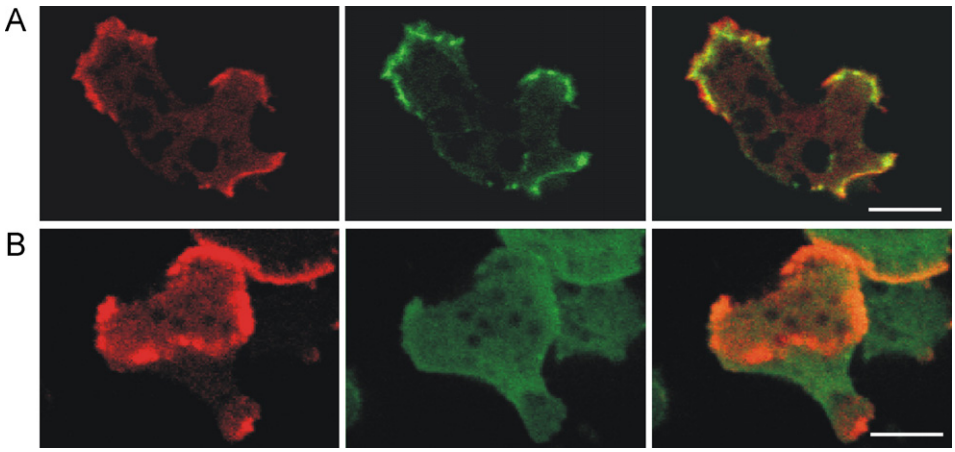

Fig. 3. Visualization of actin cytoskeleton dynamics. (A) Coexpression of mRFPmars-LimEΔ (red, left image) in combination with GFP-coronin (green, middle image) in a *Dictyostelium* cell demonstrates the sequential enrichment of cytoskeletal proteins (merge, right image). While LimEΔ, which marks filamentous actin, is localizing to the front, coronin, an actin-depolymerizing protein, enriches further back from the assembly site of actin and promotes the disassembly of F-actin. (B) A *Dictyostelium* cell coexpressing mRFPmars-LimEΔ (in red, left image) in combination with GFP-cortexillin I (in green, middle image) captured during actin wave formation. While actin is assembled in highly dynamic waves, cortexillin, an actin-bundling protein, is excluded from the waves (merge, right image). Bars, 5 μm.

pathbreaking for the identification and analysis of cell adhesion molecules (CAMs) in higher eukaryotes (Müller and Gerisch, 1978).

(2) Another ground-breaking finding was the detection of cAMP as the chemotactic agent (Konijn et al., 1967) which is produced and secreted by starving cells, sensed by neighboring cells and central to the relay system that mediates aggregation of single cells into multicellular bodies during development (Devreotes and Steck, 1979; Gerisch et al., 1975). These innovative studies provided the basis for the understanding of *Dictyostelium* chemotaxis, and its use as a model for mammalian cell chemotaxis.

(3) *Dictyostelium* amoebae are highly motile throughout their life, *i.e.* in the vegetative phase as well as during aggregation and further development. Motility is dependent on actin-driven processes (Fig. 3). Directed movement involves actin polymerization in pseudopods at the front of cells in combination with myosin-II-dependent retraction at the rear. In addition, a whole arsenal of interactors including actin-binding proteins and other regulators like protein kinases or small GTPases is needed for the regulation of cell motility (Insall and Machesky, 2009). One of the outstanding advantages of the *Dictyostelium* model system is a common repertoire of cytoskeletal proteins with higher eukaryotes. Some of them, for instance coronin, were initially described in *Dictyostelium* and only later discovered in mammals (de Hostos, 2008). Moreover, through biochemical and molecular genetic analysis of the *Dictyostelium* orthologs, the functional activities of many cytoskeletal regulators could be defined. In recent years, the combination of fluorescently labeled cytoskeletal proteins with live-cell imaging employing different microscopy techniques has revealed and visualized hitherto unappreciated details of actin cytoskeleton dynamics (Bretschneider et al., 2009, 2004) (Fig. 3).

(4) The extraordinary power of the green fluorescent protein (GFP) for the visualization of specific compartments and organelles, the localization of individual proteins and the analysis of protein dynamics was almost immediately recognized in the *Dictyostelium* field. One of the first studies that nicely emphasized the potential of the GFP-technique was live-cell imaging of GFP-coronin in *Dictyostelium* amoebae during phagocytosis of yeast cells (Maniak et al., 1995). To this day, the technique has been constantly improved and used to study a great variety of biological processes in the *Dictyostelium* model system.

(5) *Dictyostelium* mutants lacking non-muscle myosin-II revealed an unexpected essential role of this motor protein in cytokinesis and showed that it is involved in the formation of the contractile ring (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987). Later it was found that on a substratum *Dictyostelium* can complete cytokinesis even in the absence of myosin-II through an adhesion-dependent process (Neujahr et al., 1997). These and other experiments have established *Dictyostelium* as a useful model to study different aspects of cytokinesis (Fig. 4).

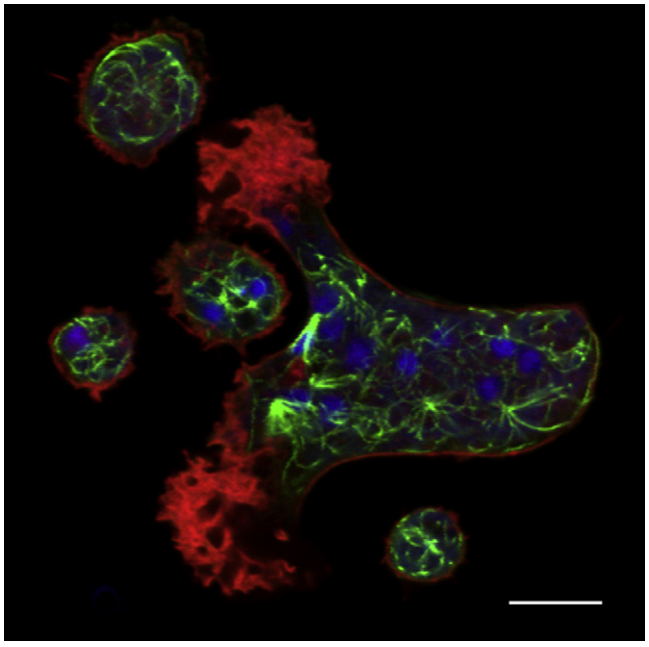

Fig. 4. Mutants lacking the actin regulators coronin and Aip1 are defective in cytokinesis. *Dictyostelium* cells lacking the actin-regulators coronin and Aip1 are multinucleate due to defects in cleavage furrow formation (Ishikawa-Ankerhold et al., 2010). Fixed coronin-Aip1-null cells were labeled for F-actin (red), α-tubulin to visualize microtubules (green) and DNA (blue). Due to impaired disassembly, F-actin enriches in cortical patches. Bar, 10 μm.

Dictyostelium and biomedical research

Dictyostelium is a genetically tractable organism, and the molecular tools available to manipulate and investigate the cells are manifold (Eichinger, 2003). One of the great advantages of the system is the ease of generating knock-out, knock-in and antisense mutants. Isogenic cells can be grown in large quantities which facilitate protein purification and biochemical studies. Furthermore, state of the art cell biological tools are available.

Despite its evolutionary distance to man, *Dictyostelium* has a great potential for addressing disease-related processes and to understand the principles underlying pathological aberrations. The usefulness of *Dictyostelium* as a model for research on human disease genes is constantly expanding and has been reviewed recently (Annesley and Fisher, 2009; Williams, 2010; Williams et al., 2006). Here just some examples with the main focus on more recent additions are presented, and documented by either original studies or reviews summarizing earlier work.

Cell migration in health and disease

The capacity to respond and to migrate directionally toward external cues is crucial for a variety of vitally important processes like angiogenesis, innate immunity, inflammatory responses, wound healing, nerve growth, and embryogenesis. Metastatic growth is often characterized by imbalances of one of these processes.

Dictyostelium cells are intrinsically motile and serve as an excellent model to analyze cell motility. Their mode of movement, called 'amoeboid', is very similar to migration of leukocytes, macrophages and some tumor cells, and governed by the same molecular principles (Charest and Firtel, 2007; Parent, 2004). The similarities of leukocytes and *Dictyostelium* have been described already decades ago (Devreotes and Zigmond, 1988), but only more recent work uncovered the details that the two systems share (Barry and Bretscher, 2010; Friedl et al., 2001). Migration of these highly motile cells is characterized by polarized cell shape, low adhesiveness, and dynamic protrusion of actin-rich pseudopods at the leading edge and retraction of the rear. The rear-end retraction is myosin-II dependent, but *Dictyostelium* cells lacking myosin-II still are still motile (Wessels et al., 1988), and display unchanged rearward traction forces at the leading edge (Iwadate and Yumura, 2008). Leukocyte migration within confined and 3D environments surprisingly also relies primarily on contractile forces and actin network expansion (Lämmermann et al., 2008). During recent years, pioneering conceptual studies employing the *Dictyostelium* system revealed intricate details of pseudopod formation and chemotactic movement (Insall, 2010; Insall and Machesky, 2009; Van Haastert, 2010). Interestingly, the formation of blebs by contraction-based internal pressure was described as an alternative way to contribute to the formation of cellular protrusions in leukocytes and *Dictyostelium* (Lämmermann and Sixt, 2009; Renkawitz and Sixt, 2010; Yoshida and Soldati, 2006).

The establishment of cellular polarity is a prerequisite for directed cell movement and regulated by complex signaling pathways. Fundamental aspects of eukaryotic chemotaxis have been deciphered first in *Dictyostelium*, for instance how binding of chemoattractants to specific G-protein coupled receptors ultimately leads to redistribution of cytoskeletal components, cell polarization, and migration of the cell (Swaney et al., 2010). Substantial similarity of the signaling pathways in *Dictyostelium* and neutrophils is nicely exemplified by the evolutionarily conserved S/T kinase complex TORC2 (target of rapamycin complex 2) which is involved in the regulation of the actin cytoskeleton in various systems (Cybulski and Hall, 2009). Consistent with the earlier findings in *Dictyostelium* (Lee et al., 2005), it has recently been confirmed that TORC2 is a key regulator of neutrophil polarity and chemotaxis (Liu et al., 2010; Liu and Parent, 2011).

Table 1
Pathogenic bacteria that can infect *D. discoideum*.

Bacterial pathogen	References
Legionella pneumophila	Hägele et al. (2000) and Solomon and Isberg (2000)
Mycobacterium avium, *M. marinum*, *M. tuberculosis*	Hagedorn and Soldati (2007), Skriwan et al. (2002) and Solomon et al. (2003)
Pseudomonas aeruginosa	Cosson et al. (2002), Pukatzki et al. (2002) and Skriwan et al. (2002)
Vibrio cholerae	Pukatzki et al. (2006)
Klebsiella pneumoniae	Benghezal et al. (2006)
Neisseria meningitidis	Colucci et al. (2008)
Burkholderia cenocepacia	Aubert et al. (2008)
Salmonella thyphimurium	Jia et al. (2009) and Sillo et al. (2011)

Modified from Bozzaro and Eichinger (2011).

Dictyostelium and innate immunity

Based on the assumption that threats to *Dictyostelium*'s survival must also occur during its development, a previously unknown cell type, now termed sentinel (S) cell, has been described (Chen et al., 2007). S cells appear to provide immune-cell like functions and were observed to engulf bacteria and sequester toxins while circulating within the slug. A Toll/interleukin-1 receptor (TIR) domain protein, TirA, was shown to be required for some S cell functions and for vegetative amoebae to feed on live bacteria. The data suggest that this apparent innate immune function and the use of TirA for bacterial feeding developed from an ancient cellular foraging mechanism that may have been adapted to serve defence functions before the diversification within the tree of life (Chen et al., 2007).

The value of non-mammalian hosts for the investigation of human microbial pathogens was only accepted after a pioneering study of the Ausubel laboratory in the mid-nineties of the last century (Rahme et al., 1995). As a soil amoeba and a professional phagocyte, *D. discoideum* feeds on bacteria and might also be a natural host of opportunistic bacteria. Furthermore, the process of phagocytosis appears to be very similar in *Dictyostelium* and macrophages (Clarke et al., 2006). Thus, *Dictyostelium*'s strategies to counteract pathogens are likely to be of general relevance (Cosson and Soldati, 2008). A landmark report in 1978 described a few bacteria that were pathogenic for *Dictyostelium* (Depraitere and Darmon, 1978). Two groups independently demonstrated more than twenty years later that *Dictyostelium* can be infected with *Legionella pneumophila* (Hägele et al., 2000; Solomon et al., 2000). These pioneering studies motivated the further use of *Dictyostelium* as a model host for *L. pneumophila* and for other human bacterial pathogens, the most recent addition to the list being *Salmonella typhimurium* (Table 1; for recent reviews see: Bozzaro et al., 2008; Bozzaro and Eichinger, 2011; Clarke, 2010; Hilbi et al., 2011; Isberg et al., 2009; Lima et al., 2011; Soldati and Neyrolles, 2012; Steinert, 2011).

Dictyostelium is an extremely powerful system for the elucidation of host cell factors during infection (Bozzaro et al., 2008; Bozzaro and Eichinger, 2011). Due to space limitations we will only present one particular beautiful study of a non-targeted approach that was used to identify *D. discoideum* mutants in which *L. pneumophila* intracellular replication was altered. One of the ten mutants that were identified in a REMI (restriction enzyme mediated integration) screen had an insertion in the *dupA* gene, encoding a putative tyrosine kinase/dual-specificity phosphatase. Inactivation of *dupA* resulted in depressed *L. pneumophila* growth and sustained hyperphosphorylation of the amoebal MAP kinase ERK1, consistent with a loss of phosphatase activity. Since MAP kinase

phosphatases are known to be highly upregulated in macrophages in response to *L. pneumophila*, the authors speculated that the DupA regulated MAP kinase response to bacteria is conserved from amoebae to mammals (Li et al., 2009).

To further exemplify the value of *Dictyostelium* for the study of host–pathogen interactions we will briefly describe recent results with *M. marinum* infected *Dictyostelium* cells. After uptake by phagocytosis, the pathogen prevents the maturation of the phagosome and replicates inside a compartment that resembles an early endosome. In a detailed analysis the proliferation phase of *M. marinum* could be divided in three distinct phases (i) an initial lag phase until 12 h post infection (hpi), (ii) a major proliferation phase from 12 to 37 hpi and (iii) a plateau or decrease in the cfu (colony forming units) after 37 hpi (Hagedorn and Soldati, 2007). They could further subdivide the major proliferation phase into four stages. In the early stage 1, a single mycobacterium resides in a vacuole enriched in vacuolin. The second stage is defined by the proliferation of the bacteria. At the late stages 3 and 4, the vacuolin-positive membrane is ruptured and bacteria are released into the cytosol (Hagedorn and Soldati, 2007). In a hallmark paper, Hagedorn et al. described that *M. marinum* and *M. tuberculosis* but not *M. avium* can spread to neighboring cells *via* a non-lytic mechanism that requires the host cytoskeleton and an intact mycobacterial ESX-1 secretion system (Hagedorn et al., 2009). This finding will surely stimulate research into the spreading of *M. tuberculosis* in mammalian cells.

Dictyostelium and the molecular basis of neurodegenerative disorders

Although *Dictyostelium* neither has a brain nor muscle it is, as exemplified below, also used to investigate the molecular basis of several neurodegenerative disorders.

Parkinson's disease (PD) is a neurodegenerative disease affecting more than five million people world wide. Recently, a number of genetic risk factors implicated in PD have been discovered. Mutations in human leucine-rich-repeat kinase 2 (LRRK2) have been found to be thus far the most frequent cause of late-onset PD (Paisan-Ruiz et al., 2004; Zimprich et al., 2004). LRRK2 belongs to the Roco family of proteins, which are characterized by the presence of a Ras-like GTPase domain called ROC, a characteristic COR (C-terminal of Roc) domain, and a kinase domain (Bosgraaf and Van Haastert, 2003). Importantly, pathogenic mutations in LRRK2 result in decreased GTPase activity and enhanced kinase activity, suggesting a gain of abnormal function (Cookson, 2010). Therefore LRRK2 kinase activity provides an interesting therapeutic target. However, due to difficulties to obtain recombinant LRRK2 protein and the subtle phenotypes of mutants in mammalian cells, detailed biochemical and structural understanding is lacking so far. *Dictyostelium* encodes 11 Roco proteins, and in particular Roco4 has the same domain structure and biochemical characteristics as LRRK2 (Marin et al., 2008; van Egmond and van Haastert, 2010). Hence *Dictyostelium* is currently used as a model to elucidate the intramolecular regulatory mechanisms of Roco proteins in order to understand LRRK2-mediated PD. The structures of *Dicyostelium* wild-type Roco4 kinase and of the mutant homologous to the human G2019S mutation were previously solved. Comparison of the structures revealed that serine 2019 of the mutant protein forms a new hydrogen bond with a conserved arginine. This locks the flexible activation loop of the kinase in the active state and explains the PD-disease related enhanced kinase activity (Gilsbach et al., 2012). *Dictyostelium* cells with a deletion of the *roco4* gene have a strong developmental defect; they are unable to make a normal fruiting body due to defective synthesis of cellulose (Fig. 5; van Egmond and van Haastert, 2010). This phenotype is rescued by expression of wild type Roco4 but also by a chimeric protein in which the Roco4 kinase domain is replaced with the kinase domain of mammalian LRRK2 (Fig. 5). Small molecule inhibitors of the kinase activity that specifically counteract the PD-mediated effects *in vivo*, may provide important clues for a treatment of PD. Roco4 kinase activity is both *in vitro* and *in vivo* inhibited by LRRK2 inhibitors (Fig. 5). Furthermore, the recently solved structure of Roco4 kinase in complex with the LRRK2 inhibitor H1152 shows that *Dictyostelium* is an excellent model system to obtain insight into the binding mechanism, the optimization of currently known and the identification of new LRRK2 inhibitors (Gilsbach et al., 2012).

p97 (VCP or valosin containing protein in mammals and Cdc48p in yeast) is a ubiquitously expressed and evolutionarily highly conserved hexameric member of the magnesium-dependent Walker P-loop AAA-ATPases. Point mutations in the human p97 gene, with R155 being a mutation hotspot, can cause autosomal-dominant IBMPFD (Inclusion Body Myopathy with early-onset Paget disease and Frontotemporal Dementia) or ALS14 (Amyotrophic Lateral Sclerosis 14) (Hübbers et al., 2007; Johnson et al., 2010). *Dictyostelium* and human p97 are 78% identical over the entire protein length. Ectopic expression of p97 as well as $p97^{R155C}$ fused to RFP in AX2 wild-type and autophagy 9 knock-out cells induced dominant negative changes and provided evidence that p97 functionally links proteasomal activity and autophagy in *Dictyostelium* (Arhzaouy et al., 2012). In a series of co-immunoprecipitation experiments using lysates from *D. discoideum*, various mammalian cell lines and tissues, the WASH (Wiskott–Aldrich Syndrome Protein and SCAR Homolog) complex subunit strumpellin (KIAA0196) was identified. Interestingly point mutations in strumpellin cause hereditary spastic paraplegia (HSP) (Clemen et al., 2010). The findings provide a link between p97 and HSP, and suggest that mutant forms of strumpellin and p97 may have a concerted pathogenic role in various protein aggregation and neurodegenerative diseases (Clemen et al., 2010, 2012). Mutations associated with spastin, another member of the AAA protein family, cause the most frequent form of HSP. To better understand its function, cell biological and biochemical analyses of the *Dictyostelium* spastin ortholog are currently being performed (A. Müller-Taubenberger and G. Woehlke (TU Munich), work in progress).

Last but not least, *Dictyostelium* is currently also being used to study Huntington disease, another neurodegenerative disorder. The disease is caused by the extension of a polyglutamine stretch in the *htt* gene encoding huntingtin (Htt) (MacDonald et al., 1993). Mammalian Htt is important for neural tube formation and brain morphogenesis; however its exact cellular function is largely unknown. Two recent studies on the single *Dictyostelium* Htt homologue highlight the potential of *Dictyostelium* to better understand the function of Htt. Myre et al. showed that disruption of the Htt gene produces cell autonomous defects that affect cAMP signaling, and as a consequence development is disturbed (Myre et al., 2011). Htt deficient cells migrated slower toward the chemoattractant cAMP and contained lower levels of cortical myosin-II, which is likely due to defects in dephosphorylation of myosin II by protein phosphatase 2A (Wang et al., 2011). It will be interesting to investigate which of these functions are conserved in mammals and how they might affect Huntington disease.

Further areas of Dictyostelium in medical research

A nice example how research using *Dictyostelium* and lymphoblasts can complement each other is a study on Shwachman–Bodian–Diamond syndrome (SBDS). This is a rare autosomal disease characterized by ineffective hematopoiesis, increased risk for leukemia and pancreatic insufficiency. Mutations within the SBDS gene, which encodes a highly conserved protein of unknown function, are associated with the syndrome. Conditional *Dictyostelium* SBDS mutant cells revealed a defect in the maturation

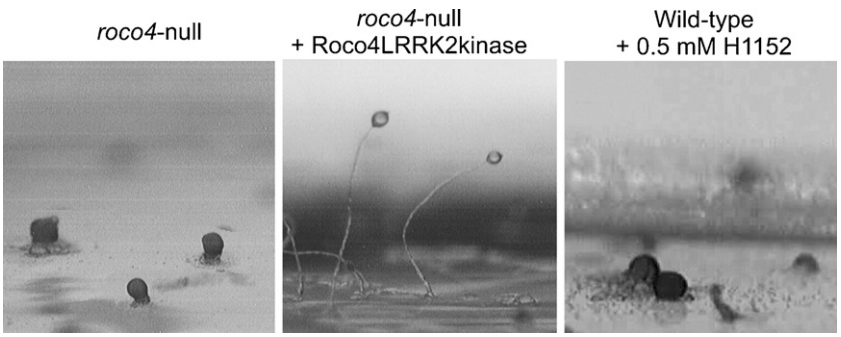

Fig. 5. LRRK2 function and inhibition experiment with *Dictyostelium* cells. *roco4*-null and *roco4*-null cells expressing Roco4, or Roco4-LRRK2 kinase in which the kinase domain of Roco4 has been replaced with the kinase domain of LRRK2, were allowed to develop for 48 h on nutrient-free agar. Pieces of agar were excised and photographed from the side. The right panel shows a side view of the development of wild-type cells in the presence of the LRRK2-inhibitor H1152. Cells in the presence of 0.5 mM H1152 have the typical *roco4*-null phenotype.
Figure modified with permission from *Proc. Natl. Acad. Sci. U. S. A.* (Gilsbach et al., 2012).

of the 60S ribosomal subunit, which is fundamental to the pathophysiology of the disorder. The findings strongly suggest that SBDS is a ribosomopathy (Wong et al., 2011).

Mitochondrial diseases are very diverse and caused by mutations affecting mitochondrial proteins (Wallace, 2010). So far the complex cytopathology of mitochondrial diseases is not well understood and usually attributed to insufficient ATP. In recent years *Dictyostelium* was established as a model for mitochondrial disease (Barth et al., 2007; Francione et al., 2011). In a very interesting publication it was reported that diverse cytopathologies were caused by chronic AMPK (AMP-activated protein kinase) signaling and not by insufficient ATP (Bokko et al., 2007). Chronic AMPK signaling in response to mitochondrial dysfunction was also found to enhance *Legionella* proliferation in the *Dictyostelium* host (Francione et al., 2009). The finding could explain the higher susceptibility of human patients with mitochondrial disease to recurrent bacterial infections (Edmonds, 2004).

Dictyostelium is also being used for pharmacological research in order to investigate the action of mood-stabilizing drugs such as lithium or valproic acid that are widely used to treat bipolar disorder and epilepsy (Chang et al., 2011; Ludtmann et al., 2011; Terbach et al., 2011; Williams et al., 2002), or of chemotherapeutic drugs employed in anti-cancer treatment (Alexander and Alexander, 2011). This list could be further extended by a number of interesting studies ranging from the investigation of the pathological processes underlying lissencephaly (Rehberg et al., 2005), and the cell biology and molecular base of lysosomal and trafficking diseases (Maniak, 2011), to the misregulation of the actin cytoskeleton causing a variety of disease pathologies, including compromised immunity, neurodegeneration, and cancer spread (Carnell and Insall, 2011).

Other key aspects for using Dictyostelium as a model

Dictyostelium plays also an important role in comparative phylogenetics and social evolution. Studies on social amoebae help to explore social interactions at physiological, genetic, and genomic levels. The social stage of *Dictyostelium* that begins with the aggregation stage of the organism (see Fig. 2) is analogous to a social group, and is thus vulnerable to internal conflict. *Dictyostelium* has become a new model for social evolution helping to understand social organization, and in particular how social cheaters are controlled (Strassmann and Queller, 2011; Strassmann et al., 2000). In these studies, cheaters were shown to be limited from exploiting other clones by mechanisms such like high relatedness, kin discrimination, pleiotropy, noble resistance, and incidental role assignment (Foster et al., 2004; Khare et al., 2009; Mehdiabadi et al., 2006). The active nature of these limits is reflected in the elevated rates of change in social genes compared with non-social genes (Santorelli et al., 2008). Despite control of cheaters, some conflict is apparent in chimeras that show slower movement of slugs (the motile aggregate formed during the multicellular stage of *Dictyostelium* development, see Fig. 2), and different contributions to stalk and spore cell populations (Kuzdzal-Fick et al., 2011).

Another unique behavior of *Dictyostelium* that has been recently reported is a primitive form of farming (Brock et al., 2011). When food becomes scarce, about one third of *Dictyostelium* clones stop feeding early and incorporate bacteria into their fruiting bodies. This has been interpreted as a way that the 'farming' *Dictyostelium* can seed a new bacterial colony as a food source in case the new habitat should be lacking edible bacteria. This genetically built-in behavior is costly for the individual, but provides benefits for the population to persist in nature. Interestingly, some amoebae carry bacteria that are not used as food, and this type of symbiosis will be central for further studies (Brock et al., 2011).

Research using *Dictyostelium* also has helped to understand the evolution of epithelial polarity in metazoans. During its multicellular stage, *Dictyostelium* forms a polarized epithelium. Epithelial polarity in metazoans requires α- and β-catenin, and homologs of both proteins are present in *Dictyostelium*. Recent data suggest that the catenin complex is the ancient functional module that mediates epithelial polarity in the absence of cadherins, Wnt-signaling components and polarity proteins (Dickinson et al., 2011).

Concluding remark

Taken together, this review throws light on the manifold topics covered by basic research using the model organism *Dictyostelium*. Even though it is impossible to cite every aspect of *Dictyostelium* research within the limits of a short review, these examples provide evidence that a lot can be learned about general biological principles by exploiting the genetic advantages and the 'simplicity' of the *Dictyostelium* system. Thus, in biosciences and in particular cell biological and biomedical research, the simple eukaryote *Dictyostelium* will continue to serve as a valuable model with great potential to investigate fundamental biological questions.

Acknowledgements

This review exemplifies just some of the landmark findings using *D. discoideum* as a model and its use to study the molecular etiology of diseases. We apologize for not having been able to consider other interesting contributions to this topic, but due to space limitations we were forced to be selective. More information

about the organism can be found at http://www.dictybase.org/. This review is based on an earlier version which was published in *Cell News* 04/2011 and has been extensively reworked and extended. We thank Dr. Hellen Ishikawa-Ankerhold (LMU Munich) for providing Figs. 1, 3 and 4. LE wants to thank his former teacher Elisabeth Fendt for putting him on the right track a long time ago. Financial support of the Michael J. Fox Foundation to AK, the Deutsche Forschungsgemeinschaft to AMT (SFB 914) and LE (SFB 670 and FOR 1228), and Köln Fortune and the Tom Wahlig Foundation (TWS) to LE is acknowledged.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejcb.2012.10.003.

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