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Microreview

Intracellular killing of bacteria: is *Dictyostelium* a model macrophage or an alien?

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Summary

Predation of bacteria by phagocytic cells was first developed during evolution by environmental amoebae. Many of the core mechanisms used by amoebae to sense, ingest and kill bacteria have also been conserved in specialized phagocytic cells in mammalian organisms. Here we focus on recent results revealing how *Dictyostelium discoideum* senses and kills non-pathogenic bacteria. In this model, genetic analysis of intracellular killing of bacteria has revealed a surprisingly complex array of specialized mechanisms. These results raise new questions on these processes, and challenge current models based largely on studies in mammalian phagocytes. In addition, recent studies suggest one additional level on complexity by revealing how *Dictyostelium* recognizes specifically various bacterial species and strains, and adapts its metabolism to process them. It remains to be seen to what extent mechanisms uncovered in *Dictyostelium* are also used in mammalian phagocytic cells.

Introduction

Phagocytosis appeared during evolution of unicellular eukaryotic organisms essentially as a way to acquire food by predating other microorganisms. In higher multicellular eukaryotes, phagocytosis allows specialized immune phagocytic cells to ingest and destroy potential pathogens. Professional mammalian phagocytes (e.g. macrophages and neutrophils) share with unicellular phagocytes (e.g. *Dictyostelium* amoebae) the ability to ingest and kill a large number of microorganisms (Steinert, 2011). They also frequently face the same virulence traits developed by bacteria in the course of evolution: bacteria largely make use of the same mechanisms to resist predation by *Dictyostelium* and by mammalian phagocytes (Cosson and Soldati, 2008).

There have been a number of excellent recent reviews dealing with the manner in which pathogenic bacteria avoid killing by *Dictyostelium* cells and mammalian phagocytes (Clarke, 2010; Bozzaro and Eichinger, 2011; Steinert, 2011; Soldati and Neyrolles, 2012). This review is focused on the situation in which bacteria show little or no pathogenicity, and succumb easily to phagocytic cells. The distinction is somewhat arbitrary: even the most innocuous bacteria can exceptionally infect and kill some individuals [e.g. fatal *Lactobacillus* infections (Kalima *et al.*, 1996)]. We designate here as 'non-pathogenic' bacteria that have a very low ability to infect mammals, and upon which *Dictyostelium* amoebae can efficiently feed. With this perspective, we are examining two emerging themes in the field of *Dictyostelium* research: which are the molecular mechanisms employed by amoebae to kill bacteria? How do amoebae recognize bacteria and adapt their physiology to optimize their feeding strategy?

Educated guesses on intracellular killing

A large number of mechanisms have been proposed to play a role in intracellular killing, based mostly on studies of mammalian phagocytic cells (Haas, 2007). These include production of toxic free radicals, control of the ionic environment, and lytic enzymes. *Dictyostelium* provides the opportunity to test how well we understand the molecular mechanisms ensuring intracellular bacterial killing. One way to address the question is to try to predict which gene products should be important for efficient intracellular killing of bacteria. It is then relatively easy to specifically inactivate the selected genes of interest in *Dictyostelium*, and to measure the ability of the resulting mutant amoebae to kill ingested bacteria. From the current knowledge of intracellular killing in mammalian cells, one may try to guess the gene products most likely to be essential for intracellular killing in *Dictyostelium*. Our best guesses initially were: free radicals, NRAMP1, and lysosomal hydrolases.

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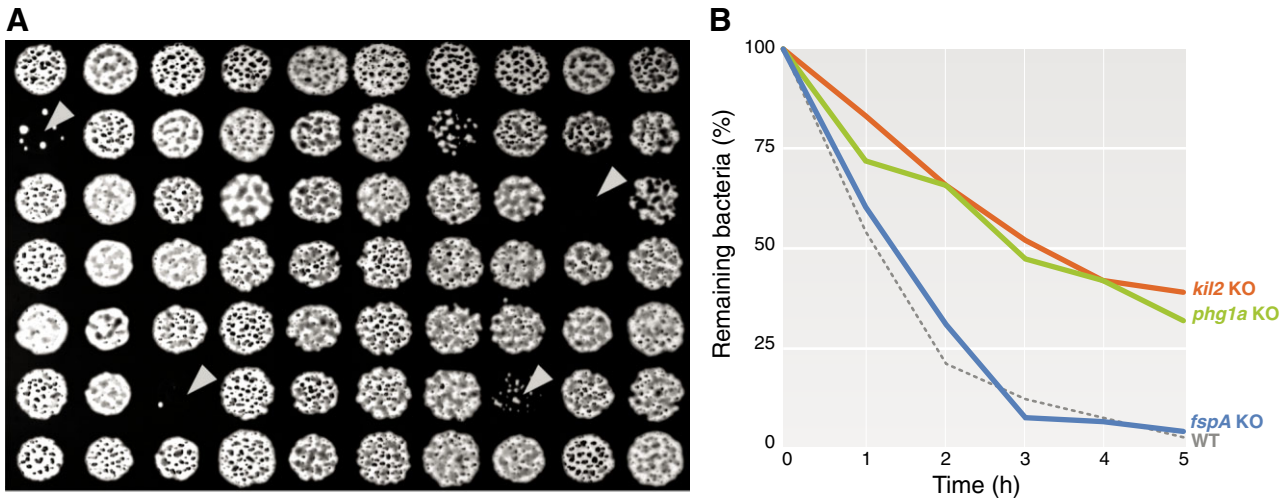

Fig. 1. Surrogate methods for measuring intracellular killing in *Dictyostelium*.
A. Growth of amoebae on a lawn of bacteria can be scaled up to allow for the screen of thousands of mutants at a time. *Dictyostelium* colonies able to feed on a lawn of *Klebsiella* bacteria (in black) form phagocytic plaques (white circles). On the contrary, mutants unable to feed on bacteria do not form such plaques (arrowheads).
B. Intracellular killing of bacteria can be more specifically measured by mixing *Dictyostelium* cells and bacteria, and assessing the number of remaining live bacteria after different times. WT and *fspA* KO cells are able to efficiently eliminate *Klebsiella* (less than 10% of bacteria remaining after 3 h), while killing-deficient mutants (as *kil2* and *phg1a* KO cells) are not (around 50% of bacteria remaining after 3 h).

Conveniently, the ability of *Dictyostelium* cells to kill various bacteria can be tentatively inferred from the ability of mutant cells to feed and grow upon various bacteria, a growth assay that allows for the testing of thousands of mutants in a simple and inexpensive way (Fig. 1A) (Froquet *et al*., 2009). However, a defective growth invites further characterization since not only intracellular killing is necessary for efficient feeding of *Dictyostelium* on bacteria, but also phagocytosis, motility, and probably bacterial sensing and metabolic adaptation (see below). A defect in intracellular killing can be characterized more specifically by measuring the survival of bacteria inside phagosomes (Fig. 1B). A non-virulent isolate of *Klebsiella pneumoniae* has been used historically to feed and grow *Dictyostelium* amoebae, and several studies have focused on the mechanisms ensuring intracellular killing of this *Klebsiella* strain, but several other non-pathogenic bacterial species are equally amenable to this type of analysis, in particular Gram-positive *Bacillus subtilis* and *Micrococcus luteus*, or Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* strains.

The protein most clearly implicated in intracellular killing of bacteria in mammals is Nox2 (or gp91^{phox}), a component of the NADPH-oxidase expressed in phagocytic cells. Nox2 is essential for the oxidative burst of phagocytic cells (e.g. neutrophils and monocytes), which is believed to play a key role in bacterial killing by free radicals (Winterbourn and Kettle, 2013). This hypothesis is based first on the observed bactericidal effect of free radicals, and second on the observation that genetic alterations of Nox2 lead to chronic granulomatous disease (CGD), a severe disorder in which patients suffer from recurrent bacterial and fungal infections (Goldblatt and Thrasher, 2000). In addition, neutrophils from mice with defective NADPH-oxidase activity kill inefficiently ingested *Staphylococcus aureus* both *in vitro* and *in vivo* (Ellson *et al*., 2006), and inhibiting the production of reactive oxygen species in human neutrophils (e.g. in hypoxic conditions) impairs intracellular killing of *S. aureus* (McGovern *et al*., 2011). More complex scenarios may be envisaged, since for example neutrophils from CGD patients are also defective for extracellular bacterial killing, as they do not produce Neutrophil Extracellular Traps (NETs), involved in binding and killing of a variety of microbes (Papayannopoulos and Zychlinsky, 2009).

The *Dictyostelium* genome contains three putative orthologues of Nox2 (NoxA, B and C), although only NoxA is expressed in vegetative cells (the other two isoforms are expressed during developmental stages) (Lardy *et al*., 2005; Bedard *et al*., 2007). While genetic inactivation of *noxA*, *noxB* or *noxC* causes altered multicellular development of *Dictyostelium* (Lardy *et al*., 2005), neither a *noxA*-null nor a *noxA*/*noxB* double null mutant show any defect in their ability to feed upon a wide variety of bacterial species, or in their ability to kill ingested *Klebsiella* bacteria (Lardy *et al*., 2005; Benghezal *et al*., 2006). Apparently, in *Dictyostelium* generation of superoxide by the NADPH oxidases NoxA and B plays mostly a role in signalling and is dispensable for efficient killing of

Klebsiella. It remains to be seen if this result would hold true in different conditions (as in a triple *noxABC* KO), or when the killing of other bacterial species is considered.

Another protein potentially involved in intracellular bacterial killing is NRAMP1, a metal ion transporter present in the phagosomal membrane of mammalian macrophages (Nevo and Nelson, 2006) and of *Dictyostelium* (Peracino *et al*., 2006). It has been shown that NRAMP1 is essential for the ability of mice to efficiently kill *Mycobacterium bovis* and *Salmonella typhimurium* (Vidal *et al*., 1995; White *et al*., 2005). NRAMP1 uses the proton gradient generated by the activity of the vacuolar H^+-ATPase to transport manganese and iron out of the phagosome, generating a metal-ion-depleted environment unfavourable for survival and replication of bacteria (Soldati and Neyrolles, 2012). Indeed, *nramp1* KO *Dictyostelium* cells allow more efficient intracellular replication of *Mycobacterium avium* and *Legionella pneumophila*. These cells do not however exhibit any defect in their ability to feed upon or to kill non-pathogenic *Klebsiella* bacteria (Lelong *et al*., 2011).

Lysosomal hydrolases may also be responsible for the degradation and killing of different microbial species (Kornfeld and Mellman, 1989). For example, genetic inactivation of cathepsin G in mice renders them more sensitive to *S. aureus* infections, and this is paralleled by a decrease in the ability of neutrophils to kill these pathogens (Reeves *et al*., 2002). In mammals, other peptidases, as elastase and proteinase 3, are involved in the conversion to the active form of antimicrobial peptides (as cathelicidins), which are able to efficiently kill many bacteria *in vitro* and *in vivo* (Zanetti, 2005).

The *Dictyostelium* genome exhibits a large array of enzymes expected to hydrolyse carbohydrates (such as lysozymes and β-hexosaminidases) and proteins (as cathepsins) (Table 1), and many of them have been localized by proteomics to the phagosomal compartment (Gotthardt *et al*., 2002; 2006; Boulais *et al*., 2010; Journet *et al*., 2012). To date the best-characterized *Dictyostelium* lytic enzyme is AlyA, which belongs to a new family of amoeba lysozymes with low levels of similarity to metazoan lysozymes. AlyA is responsible for almost 50% of the total cellular lysozyme activity, and *alyA* KO cells cannot grow efficiently upon non-pathogenic bacteria (*E. coli*, *Klebsiella* and *Micrococcus*). However, with successive passages in bacterial lawns, mutant cells recover their ability to grow upon bacteria, an effect linked to increased phagocytosis (Muller *et al*., 2005). Altogether, these observations provide no direct evidence for a role of AlyA in killing, and instead uncover an unexpected link between lysozyme activity and the regulation of phagocytosis. Clearly, cells with a decreased lysozyme activity are eventually able to compensate for this loss, pointing to the existence of other killing mechanisms. It is possible that combining *alyA* knockout with other mutations will reveal better its specific role in bacterial killing.

Cathepsins are another important group of lysosomal hydrolases involved in protein degradation and recycling, and playing a role in several physiological processes in mammalian organisms, as antigen presentation, bone remodelling and hormone processing (Reiser *et al*., 2010), besides their well-established role in degrading bacterial wall components and virulence factors (Thorne *et al*., 1976; Carrasco-Marin *et al*., 2009; Flannagan *et al*., 2009). Cathepsin D is one of the most abundant proteases in mammalian lysosomes (Kato *et al*., 1972), and it is involved in killing of *Streptococcus*, *Mycobacterium* and *Listeria* (del Cerro-Vadillo *et al*., 2006; Bewley *et al*., 2011). *Dictyostelium* genome possesses more than 30 genes annotated as cathepsins or cysteine proteases. Cathepsin D is also a major marker for *Dictyostelium* lysosome maturation, but disruption of the gene does not impair the ability of cells to feed upon *Klebsiella* (Journet *et al*., 1999).

Affecting proteins responsible for phagosome maturation may also be expected to impair bacterial killing. *Dictyostelium* WASH protein, as other metazoan orthologues, has been implicated in vesicular trafficking and phagosome maturation (Carnell *et al*., 2011; King *et al*., 2013). Cells lacking WASH exhibit impaired phagosomal proteolysis and reduced amounts of lysosomal hydrolases (such as cathepsins and lysozymes), yet their growth on non-pathogenic *Klebsiella* and *Bacillus* is not affected, suggesting that their killing activity is not significantly reduced. In contrast, they grow inefficiently on several other bacterial strains or species [e.g. a pathogenic encapsulated KP52145 *Klebsiella*, or an attenuated quorum-sensing deficient *P. aeruginosa* strain (King *et al*., 2013)]. The ability of the *wash* mutant cells to kill ingested bacteria was not directly measured.

Another *Dictyostelium* protein directly involved in lysosome maturation is LvsB, an orthologue of the mammalian lysosomal trafficking regulator LYST. In *Dictyostelium*, as in mammalian cells, this protein regulates lysosome biogenesis, acidification and secretion (Cornillon *et al*., 2002; Harris *et al*., 2002; Charette and Cosson, 2007; Kypri *et al*., 2007). As seen for *wash* KO cells, *lvsB* KO cells also have specific growth defects: they are unable to feed upon *M. luteus* and some pathogenic *Klebsiella* strains, but can grow as efficiently as WT cells on other bacteria, as *Bacillus* and *E. coli*.

Overall, the general conclusion of these various attempts is that no single gene product targeted so far played a crucial role in intracellular killing of ingested non-pathogenic bacteria. This may be due to the presence of redundant killing mechanisms, or to the fact that the most important gene products were not tested.

Table 1. Role of various gene products in bacterial sensing and killing in *Dictyostelium*.

	Gene	Dictybase ID	Molecular identity	KO phenotype – mammalian[a]	KO phenotype – *Dictyostelium*	Reference
Candidates by analogy with mammalian system	*alyA*	DDB_G0275123	Lysozyme	NA[b]	– Defective growth on non-pathogenic *Klebsiella* – 50% reduction of lysozyme activity – Normal killing of non-pathogenic *E. coli*	Muller *et al.* (2005)
	catD	DDB_G0279411	Cathepsin D	– Impaired killing of *Listeria*, *Streptococcus* and *Mycobacterium* – Accumulation of undigested material on lysosomes	– Normal growth on non-pathogenic *Klebsiella*	Journet *et al.* (1999)
	lvsB	DDB_G0271504	LYSosomal Trafficking regulator (LYST) homologue	– Defective lysosome maturation and secretion – Defective antigen processing and presentation – Decreased cytotoxic killing ability of T lymphocytes, NK cells, and granulocytes	– Defective lysosome maturation and acidification – Defective lysosome enzymes content and processing – Defective growth on *S. aureus*, *M. luteus* and pathogenic *Klebsiella*, but not on *B. subtilis*, *E. coli* and non-pathogenic *Klebsiella*	Cornillon *et al.* (2002); Harris *et al.* (2002)
	nox2	DDB_G0289653	NADPH oxidase, large subunit	– Recurrent infections by *S. aureus*, *Salmonella*, *Klebsiella*, *Aerobacter* and *Serratia* in human CGD patients – Impaired killing of *S. typhimurium*, *Burkholderia pseudomallei* and *K. pneumoniae* by mice and rat macrophages	– Normal killing of *Klebsiella* – Normal growth on non-pathogenic *E. coli* and *Klebsiella*	Lardy *et al.* (2005)
	nramp	DDB_G0276973	Fe^{3+}/Mn^{2+} transporter	– Impaired killing of *Salmonella* and *Mycobacterium* by mice macrophages	– Impaired killing of *Legionella* and *Mycobacterium*, but not of *Klebsiella* – Normal growth on *Klebsiella*	Peracino *et al.* (2006)
	wshA	DDB_G0292878	WASP and SCAR homologue	– Defective endosomal trafficking, maturation and sorting	– Defective growth on *E. coli*, *S. aureus*, *M. luteus* and pathogenic *Klebsiella*, but not on *B. subtilis* and non-pathogenic *Klebsiella* – Defective lysosome maturation, neutralization and secretion – Defective lysosome proteolysis and hydrolases content	Carnell *et al.* (2011)
Candidates by random mutagenesis	*fspA*	DDB_G0277237	Putative GPCR-like protein	NA	– Defective growth on non-pathogenic *E. coli* and *Klebsiella* – Normal killing of *Klebsiella* – Defective chemokinetic activation by *Klebsiella*	Lima *et al.* (2014)
	kil1	DDB_G0267630	Sulfotransferase	NA[c]	– Defective killing of *Klebsiella*, but not of *B. subtilis* – Normal growth on non-pathogenic *E. coli* and *Klebsiella*	Benghezal *et al.* (2006)
	kil2	DDB_G0279183	Type V Mg^{2+} P-ATPase	– Impaired lysosomal biogenesis and acidification and decreased lysosome proteolysis	– Defective killing of *Klebsiella*, but not of *B. subtilis* – Defective growth on non-pathogenic *E. coli* and *Klebsiella*	Lelong *et al.* (2011)
	phg1a	DDB_G0267444	TM9-family protein	– Impaired phagocytosis of neighbouring cells by cannibalistic melanoma	– Defective killing of *Klebsiella*, but not of *B. subtilis* – Defective growth on *E. coli* and *Klebsiella*	Cornillon *et al.* (2000); Lozupone *et al.* (2009)
	tirA	DDB_G0289237	TIR domain-containing protein	NA[d]	– Defective growth on non-pathogenic *Klebsiella* – Susceptibility to non-pathogenic *Legionella*	Chen *et al.* (2007)

a. NA: no KO available for the homologous gene in mammalian systems.
b. Lysozymes have primarily a bacteriolytic function, and are involved in bacterial killing and immune response in general.
c. In mammalian cells, sulfation reactions play a role on epitope generation for ligands of cell adhesion receptors, although no role on bacterial sensing and killing has been described to date.
d. TIR domains in mammalian proteins (as MyD88, interleukin-1 receptor and TLRs) are involved in interactions between the TLRs and signal-transduction components.

Dictyostelium provides a convenient model to inactivate specific genes and assess their role in intracellular killing.

Obscure words from the slime

Another way to test the depth of our knowledge of intracellular killing mechanisms, and to simultaneously test if functional redundancy prevents *Dictyostelium* mutants from exhibiting killing defects, is to isolate randomly killing-deficient mutants, and try to make sense of the gene products identified in this manner. The first killing-deficient mutant was identified serendipitously: *phg1a* KO cells, initially characterized as defective in adhesion to and ingestion of latex beads (Cornillon *et al*., 2000), were later found to also kill inefficiently ingested *Klebsiella* bacteria (Benghezal *et al*., 2006). This defect presumably accounts for the inability of *phg1a* KO cells to feed and grow upon *Klebsiella* bacteria. In the same study, Kil1 was identified as a high-copy suppressor of the killing defect *phg1a* KO cells, and *kil1* KO cells were shown to kill inefficiently *Klebsiella* bacteria. The role of Phg1a in intracellular killing is probably due to the fact that it controls intracellular transport and stability of membrane proteins (Froquet *et al*., 2012), and that in its absence the Kil1 protein is unstable and virtually depleted from cells (Le Coadic *et al*., 2013) (Fig. 2). Kil1 is a sulfotransferase, and no direct link has previously been established between sulfation of host proteins and host–pathogen interactions in metazoans. Sulfation has been described to play a role in receptor–ligand interactions (Hemmerich and Rosen, 2000; Park *et al*., 2010), but its role in intracellular killing remains to be determined.

A random screen for *Dictyostelium* mutants identified Kil2 as another essential gene for efficient growth on *Klebsiella*. Kil2 is a putative magnesium transporter in the phagosomal membrane, and its absence leads to diminished activity of phagosomal proteases, and to inefficient intracellular killing of *Klebsiella* (Lelong *et al*., 2011). Both killing and proteolytic activity are restored by supplementation with magnesium, indicating that ionic homeostasis inside the phagosome is essential for proper hydrolytic activity and efficient bacteria processing. The role of the ionic composition of phagosomes in bacterial killing is discussed nicely in a recent review (Soldati and Neyrolles, 2012). Kil2 belongs to the group V Ptype ATPase family, and no member of this family has been previously implicated in host–pathogen interactions, nor has magnesium been previously linked to intraphagosomal killing mechanisms.

It is remarkable how little we can comment about these findings at this stage, or try to delineate mechanistic relationships among them and with mammalian gene products implicated in killing. Phg1, Kil1 and Kil2 have potential orthologues in human (Table 1), but none of them have been linked previously to host–pathogen interactions. Another important observation is that although these three gene products are necessary for intracellular killing of *Klebsiella*, they are dispensable for efficient killing of non-pathogenic *P. aeruginosa* or of Gram-positive *B. subtilis*. This suggests that *Dictyostelium* cells may use several independent mechanisms to kill different bacterial species or strains.

The taste of bugs

In animals, the recognition of invading microorganisms is essential to trigger an adequate antibacterial response and a successful defence against infections. Several proteins involved in the recognition machinery have been identified, most notably Toll receptors in *Drosophila* fruit flies (Lemaitre *et al*., 1996). The discovery and

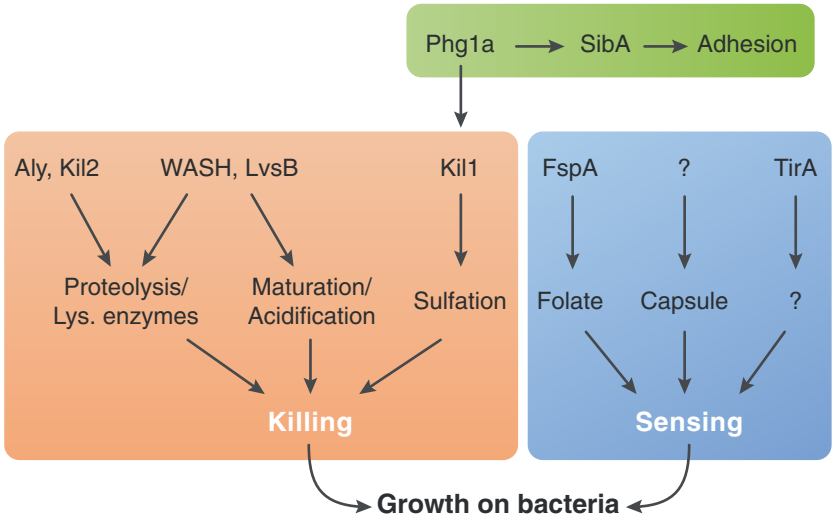

Fig. 2. Molecular mechanisms involved in *Dictyostelium* sensing and killing of bacteria. Sensing of *Klebsiella* bacteria involves different players, notably FspA for bacteria-secreted folate, and a yet-unknown receptor of capsule components. TirA may also play a regulatory role in sensing. Mechanisms related to intracellular killing have been more extensively unravelled. Lysosomal activity (as denoted by the proteolytic efficiency inside the phagosome) and phagosomal biogenesis (including proper acidification and maturation) are major factors implicated in efficient killing. Proper regulation of adhesion and sulfation processes has also been implicated in successful killing.

characterization of these receptors was the first step in the elucidation of the different molecular pathways involved in the recognition of distinct bacterial MAMPs (microbial-associated molecular patterns) (Brennan and Anderson, 2004). Today, the Toll-like receptor (TLR) family has been extensively characterized in mammals, and each member has been linked to recognition of specific microbial components, such as lipopolysaccharides, peptidoglycans or nucleic acids. TLRs are the paradigm for differential pathogen recognition in metazoans (Hoffmann and Reichhart, 2002; Akira *et al*., 2006), together with several other membrane and cytoplasmic receptors. Cytosolic NOD receptors have been implicated in the recognition of peptidoglycan and flagellin; C-type lectin receptors can recognize lipopolysaccharides, capsule polysaccharides and glycolipids; and scavenger receptors can detect lipopolysaccharides, lipoteichoic acid and several bacterial proteins (Pluddemann *et al*., 2011).

In multicellular hosts, recognition of potentially harmful microorganisms can easily be distinguished from response to other physiological signals. Ideally it should elicit danger signals that ultimately allow the host to eliminate invading pathogens. The distinction may be subtler in amoebae, for which microorganisms are both a source of food and potential pathogens. Theoretically, to successfully feed upon bacteria, amoebae may need to sense bacterial factors and migrate towards them, and to adapt their physiology to optimize ingestion, killing and digestion of the available bacteria. Ideally, they should also be able to recognize pathogenic bacteria and avoid them. These largely speculative considerations have essentially not been tested so far, most probably due to the reduced number of studies addressing these or related issues. The *Dictyostelium* genome exhibits no clear Toll-like receptors, but several putative orthologues to other bacterial-sensing receptors (Cosson and Soldati, 2008), the function of which remains to be established. We describe below this nascent field of research, stemming from a few recently published studies.

Several large-scale transcriptional studies have been conducted to analyse changes in gene expression when *Dictyostelium* cells are exposed to different bacteria, e.g. *E. coli*, *Pseudomonas* and *Legionella* (Benghezal *et al*., 2006; Farbrother *et al*., 2006; Carilla-Latorre *et al*., 2008; Sillo *et al*., 2008; Nasser *et al*., 2013). These studies clearly demonstrate that *Dictyostelium* cells confronted to different bacterial species exhibit very different gene expression profiles. However, it is not clear whether these differences are caused by specific recognition of bacteria by amoebae, or result from a long-term metabolic adaptation of *Dictyostelium* to various food sources.

One founding study on bacterial sensing identified TirA, a cytosolic TIR-domain containing protein whose eukaryotic orthologues are involved in specific antimicrobial response, and showed that its genetic inactivation rendered *Dictyostelium* cells unable to feed upon *Klebsiella* (Chen *et al*., 2007). However, up to now it is not clear if and how this protein is involved in bacterial recognition or subsequent downstream pathways.

Another study (Nasser *et al*., 2013) also identified by random mutagenesis genes essential for growth on various bacteria. The ability of amoeba cells to feed upon Gram(+) bacteria appears to be dependent on proteins involved in bacterial cell wall breakdown and N-glycosylation (probably by modulating the activity of yet-unknown membrane receptors), while response to Gram(−) bacteria appears to involve AlyL lysozyme activity, in conjunction with the Spc3 signal peptidase (which has been proposed to act as a regulator of lysozyme biogenesis). The exact role of these proteins in bacterial sensing remains to be established. It is likely that the vast majority of gene products involved in bacterial recognition by amoebae, in particular specific receptors, are still not identified today.

In a recent study we made use of the observation that exposure of *Dictyostelium* to bacteria increases their motility to show that *Dictyostelium* cells can recognize different types of bacteria, and that recognition of *Klebsiella* involves at least two distinct pathways: one sensing the folate produced by bacteria, the other one responding to bacterial capsule (Lima *et al*., 2014) (Fig. 2). Moreover, we also identified one molecular component of the folate-sensing pathway: FspA, a putative G-protein coupled receptor (GPCR) protein that may act as a receptor or a regulator in this specific sensing pathway.

Collectively, these studies support the idea that *Dictyostelium* amoebae do possess several specific bacterial-sensing mechanisms, and that these mechanisms are essential to ensure efficient growth in the presence of bacteria.

Conclusion

Dictyostelium discoideum has been used as a powerful model to study cell motility and phagocytosis, and the underlying mechanisms have proven largely similar to those identified in mammalian cells. There is no clear evidence today that *Dictyostelium* and mammalian cells use similar mechanisms to kill ingested microorganisms. Mostly, this ambiguity results from the fact that our understanding of killing mechanisms is very incomplete in these two different cell types. Recognition of bacteria by phagocytic cells may also be a prerequisite for efficient intracellular killing, and only very few studies have analysed this function in *Dictyostelium*. It is likely that, as our knowledge progresses, we will be able to distinguish which sensing and killing mechanisms are conserved

between amoebae and mammalian phagocytes, and which ones are specific for each system.

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