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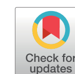
Phagocytosis in *Drosophila*: From molecules and cellular machinery to physiology



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A B S T R A C T

Phagocytosis is an evolutionarily conserved mechanism that plays a key role in both host defence and tissue homeostasis in multicellular organisms. A range of surface receptors expressed on different cell types allow discriminating between self and non-self (or altered) material, thus enabling phagocytosis of pathogens and apoptotic cells. The phagocytosis process can be divided into four main steps: 1) binding of the phagocyte to the target particle, 2) particle internalization and phagosome formation, through remodelling of the plasma membrane, 3) phagosome maturation, and 4) particle destruction in the phagolysosome. In this review, we describe our present knowledge on phagocytosis in the fruit fly *Drosophila melanogaster*, assessing each of the key steps involved in engulfment of both apoptotic cells and bacteria. We also assess the physiological role of phagocytosis in host defence, development and tissue homeostasis.

1. Introduction

Phagocytosis is defined as the cellular uptake of particles bigger than 0.5 μm through the formation of a membrane derived vesicle known as the phagosome. Its first description dates back to over 100 years ago, when the Russian zoologist Elie Metchnikoff observed that specific cells in starfish larvae were able to engulf foreign objects. This very simple observation turned out to be a crucial milestone in the history of cellular innate immunity. Phagocytosis is an ancient and evolutionarily conserved process performed by unicellular organisms and different metazoan cell types. In the former case, phagocytosis represents an important feeding mechanism (Cosson and Soldati, 2008). In higher organisms, instead, phagocytosis is mediated by dedicated cells, called phagocytes, which are able to digest both pathogens and "altered-self" particles. In this way, phagocytosis not only represents a crucial first line of defence but, importantly, also mediates tissue homeostasis via the clearance of apoptotic and necrotic cells. Despite major breakthroughs made after its discovery, the detailed molecular mechanisms underlying phagocytotic processes remain poorly understood. Mammalian systems are characterized by highly complex and redundant phagocytic components, which complicates addressing the specific role of each protein. Therefore, in the last decades, researchers have expanded their studies of phagocytosis to genetically tractable model organisms, such as the fruit fly *Drosophila melanogaster* (*Drosophila*). *Drosophila* relies entirely on the innate immune response to fight infections (Lemaitre and Hoffmann, 2007). It has a less redundant genome, which has been fully sequenced and extensively annotated, and exhibits powerful genetic and molecular techniques. These reasons made *Drosophila* an attractive and suitable model system to study the complex process of phagocytosis. The main objective of this article is to provide an up-to-date review of phagocytosis in this insect. After describing the nature of the cells that are capable of phagocytosis, we describe phagocytic receptors and opsonins that initiate the process of engulfment as well as the cellular machinery that internalizes particles and leads them to destruction. In the second part of this review, we analyse how phagocytosis contributes to development, maintenance, and host defence at the organismal level.

2. Professional and non-professional phagocytes in *Drosophila*

Different cell types have been reported to engulf foreign materials in *Drosophila*. In particular, we can distinguish between professional (globally called macrophages) and non-professional phagocytes (tissue-resident neighbouring cells). *Drosophila* possesses specialized hemocytes (i.e. blood cells) named plasmatocytes or macrophages that function as professional phagocytes. They engulf pathogens, apoptotic cells and dendrite debris. In addition to their phagocytic tasks, plasmatocytes also produce antimicrobial peptides, clotting factors, cytokines, and extracellular matrix components making them important players in the fruit fly immune response (Martinek et al., 2008;

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Samakovlis et al., 1990; Theopold et al., 2014; Vanha-aho et al., 2016). Non-professional phagocytes are usually tissue-resident cells, which in addition to their established tasks can also engulf foreign particles, in environments where circulating macrophages are less accessible. However, non-professional phagocytes frequently display decreased phagocytic abilities compared to macrophages, with respect to the variety and efficiency of particles they can take up. Nevertheless, the recognition and signalling machinery appear similar in both cell types. Several cell types have been shown to function as non-professional phagocytes in *Drosophila* (Shklover et al., 2015a), mainly mediating apoptotic cell clearance (a process also described as efferocytosis). Glial cells are one of the best characterized non-professional *Drosophila* phagocytes which mediate the shaping of the central nervous system (CNS) in developing embryos. Ovarian follicle epithelial cells are another well studied example of non-professional phagocytes. In particular, they mediate efferocytosis of nurse cells inside the egg chamber during *Drosophila* oogenesis (Serizier and McCall, 2017).

3. Phagocytic receptors and opsonins in *Drosophila*

Phagocytosis is usually initiated by dedicated receptors that bind to molecules exposed on the surface of pathogens or apoptotic cells. Well-established microbial associated molecular patterns (MAMPs) recognized by phagocytic receptors are bacterial peptidoglycans, lipopolysaccharides (LPS), or fungal β-1,3 glucans. Apoptotic cells are often decorated by phosphatidylserine (PS), a membrane phospholipid species usually found in the inner leaflet of the plasma membrane, but exposed on the surface of cells undergoing apoptosis (Segawa and Nagata, 2015). Upon ligand recognition, phagocytic receptors directly or indirectly engage downstream signalling pathways that initiate the uptake of the particle. Studies have identified a plethora of receptors with a putative role in phagocytosis, based on their similarity with engulfment receptors in other species. While some may be true phagocytic receptors, others may indirectly affect phagocytosis or be involved in the downstream steps. In the following paragraphs, we provide a critical summary of the main phagocytic receptors and potential opsonin molecules functioning in bacterial phagocytosis and apoptotic cell clearance in *Drosophila* (Fig. 1).

3.1. Scavenger receptors

Scavenger receptors (SRs) are transmembrane proteins expressed by both invertebrate and mammalian professional phagocytes. Their activity was first identified in mammalian macrophages where they endocytose modified low-density lipoprotein (mLDL) (Brown and Goldstein, 1983). In addition, SRs are also able to bind to polyanionic ligands and function as pattern recognition receptors (PRRs), mediating phagocytosis of microbes and dying cells in many species. One of the first SRs discovered in *Drosophila* is class C Scavenger receptor I (dSr-CI). The class C Scavenger receptor family counts four members in *Drosophila* (dSr-CI, dSr-CII, dSr-CIII, dSr-CIV). dSr-CI is specifically expressed on plasmatocyte surfaces and was shown to possess a similarly wide ligand recognition spectrum as mammalian class A SR (Pearson et al., 1995). *In vitro* studies demonstrated its importance in bacteria binding and phagocytosis of both Gram-positive and Gram-negative bacteria, but not yeast (Pearson et al., 1995; Rämet et al., 2001). Moreover, after bacterial challenge, transcription of *dSr-CI* is upregulated, further supporting its role in host defence (Irving et al., 2005). Interestingly, the *dSr-CI* locus presents a high polymorphism, likely a consequence of positive selection (Lazzaro, 2005). This feature could be the consequence of an arms race as often observed for host-pathogen interactions. However, the role of dSr-CI in bacterial phagocytosis has never been properly confirmed with the use of null mutations.

The *Drosophila* genome encodes twelve class B SRs with homology to the mammalian CD36, which was initially discovered in apoptotic cell clearance (Savill et al., 1992). Many of the class B SRs are expressed in the fly gut, but their function is poorly characterized (Herboso et al., 2011). One CD36 member, Croquemort (Crq), is specifically expressed in plasmatocytes and has been shown to function as a receptor for apoptotic cells (Franc et al., 1999, 1996). However, the molecular mechanisms underlying its action and its cognate ligand on apoptotic cells are still unknown. Further *in vitro* work in *Drosophila* S2 macrophage-like cells has revealed the involvement of Crq in *Staphylococcus aureus* recognition and uptake (Stuart et al., 2005). Adult *crq* knockout flies show protracted and increased *Diptericin* and *Unpaired3* expression during microbial infections and under unchallenged conditions, suggesting that Crq might indirectly alter the humoral response (Guillou et al., 2016). Nevertheless, the role of Crq as an engulfing receptor has been recently challenged by several subsequent studies, which revealed a contribution of Crq in phagosome maturation rather than in particle recognition (Guillou et al., 2016; Han et al., 2014; Meehan et al., 2016). Finally, RNAi-mediated silencing of *crq* in plasmatocytes was shown to block lipid uptake, suggesting that Crq may be involved in the acquisition of lipids, as shown for other CD36 members (Woodcock et al., 2015). Considering that lipid metabolism plays a key role in various aspects of phagocytosis (e.g. phagosome formation and maturation) (Yeung et al., 2006), it cannot be ruled out that Crq involvement in lipid scavenging might indirectly affect the phagocytic process. Several RNAi based screens on S2 *Drosophila* cells have identified another class B SR, Peste, as specifically required for the engulfment of *Mycobacterium fortuitum* and *Listeria monocytogenes*, but not *Escherichia coli* or *S. aureus* (Agaisse et al., 2005; Philips et al., 2005). The lack of *Peste* null mutants did not allow to validate these RNAi studies and fully test the relevance of this receptor in host defence.

3.2. Peptidoglycan recognition proteins (PGRPs)

PGRPs form a family of pattern recognition receptors involved in the detection of the bacterial cell wall component peptidoglycan (PGN). Some PGRPs possess enzymatic activities (amidase), cleaving peptidoglycans into non-immunogenic fragments (Mellroth et al., 2003). Other non-catalytic PGRPs can bind peptidoglycan but cannot cleave it as they lack a critical cysteine in the catalytic pocket. In insects, these non-catalytic PGRPs function as pattern recognition receptors dedicated to the identification of bacteria upstream of Toll and Imd pathways, two NF-kB pathways involved in the regulation of antimicrobial peptide genes (Royet et al., 2011). Early RNAi screens in S2 cells identified PGRP-LC as a key molecule involved in the uptake of Gram-negative, but not Gram-positive bacteria (Rämet et al., 2002). PGRP-LC is a pattern recognition receptor that recognizes DAP-type peptidoglycan found in the wall of Gram-negative and certain Gram-positive (*Bacillus*) bacteria (Stenbak et al., 2004). In contrast to the initial results obtained by RNAi, following studies using either RNAi approaches (Kocks et al., 2005) or *PGRP-LC* null mutant (Melcarne et al., unpublished), failed to confirm a major role of PGRP-LC in the phagocytosis of Gram-negative bacteria.

3.3. Nimrods

An interesting and relatively newly discovered family of phagocytic receptors in *Drosophila* comprises proteins characterized by the presence of the so-called Nimrod (NIM) repeats. The NIMs are a subtype of the epidermal growth factor (EGF) repeat, which often function in adhesion, coagulation, and receptor-target interactions (Bork et al., 1996). The *Nimrod* gene family comprises twelve members, ten of which cluster on the second chromosome, and two, *eater* and *draper*, on the third chromosome. According to their domain structure, proteins belonging to this family can be classified into different categories. The *draper*-type genes *(NimA* and *draper*) encode proteins that carry only one NIM domain, followed by several EGF repeats, and one Emilin (EMI) domain at their N-terminal end (Callebaut et al., 2003). The Draper protein was initially identified in a genetic screen as being

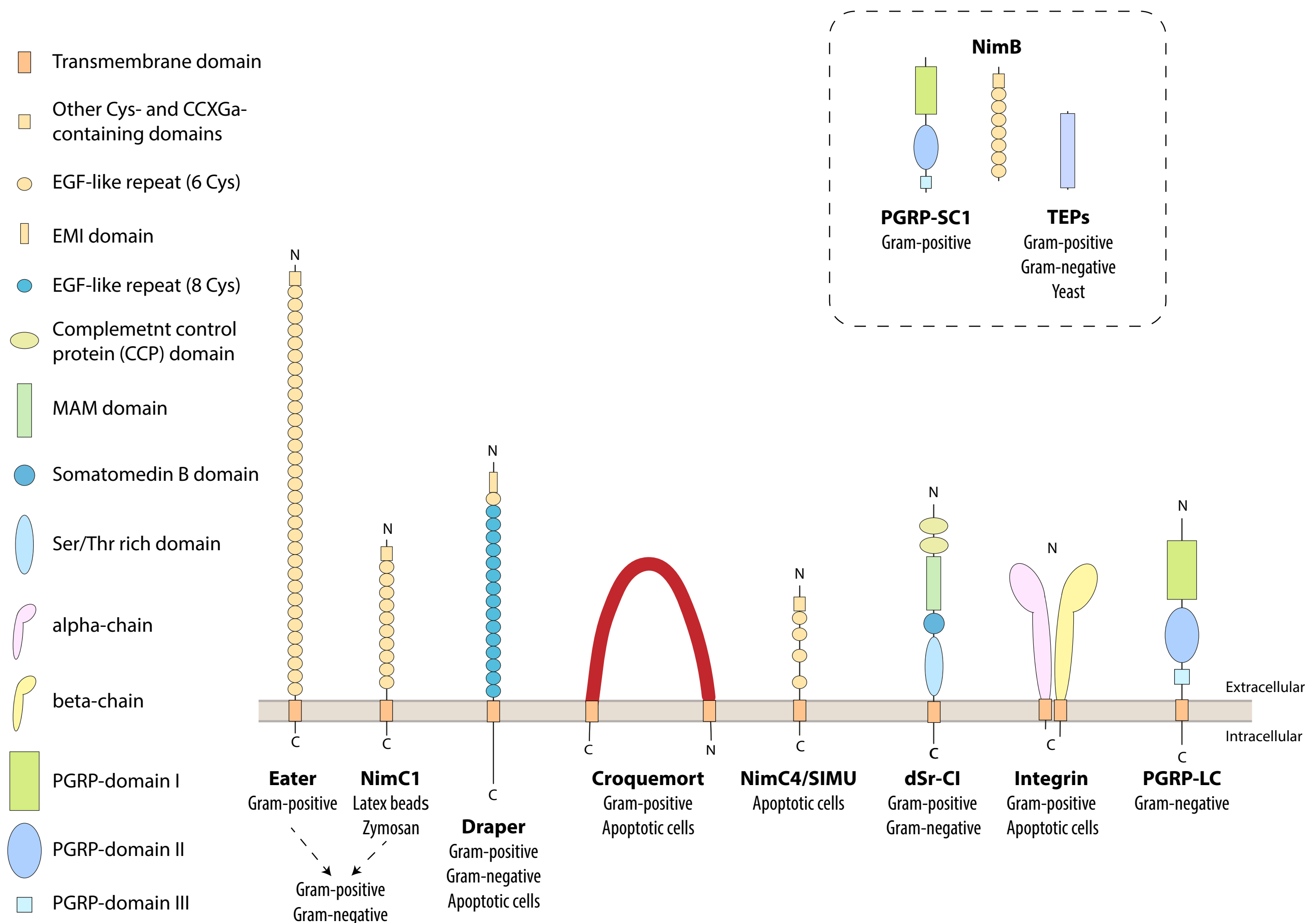

Fig. 1. Plasma-membrane phagocytic receptors and opsonins in *Drosophila*.
Graphical illustration of the main *Drosophila* phagocytic receptors involved in microbe engulfment and efferocytosis. The role of Eater, NimC1, Draper, NimC4/SIMU, PGRP-LC and Crq has been extensively characterized by using null mutants, the function of dSr-CI and Integrin was analysed solely by RNAi silencing (See main text for further details). The dashed box represents potential secreted opsonins mediating phagocytosis in *Drosophila*. Dashed arrows on Eater and NimC1 indicate the synergistic action of those receptors in bacterial phagocytosis.

regulated by Glial Cells Missing (Gcm), a key glial transcription factor (Freeman et al., 2003). It is expressed on the surface of *Drosophila* plasmatocytes and several non-professional phagocytes, such as glia and epithelial cells. Draper possesses 15 extracellular EGF repeats, a single transmembrane domain, and a signalling cytosolic tail. Draper has been reported to recognize apoptotic cells by interacting with either phosphatidyl serine (PS) or the endosomal protein Pretaporter, that are found on the surface of dying cells (Kuraishi et al., 2009; Tung et al., 2013). During development, Draper mediates not only the removal of dying neurons, but it is also involved in neuronal pruning and synapse clearance (Awasaki et al., 2006; Kurant et al., 2008; Logan et al., 2012; Manaka et al., 2004; Ziegenfuss et al., 2008). Moreover, it plays a key role in nurse cell clearance by epithelial follicle cells in the *Drosophila* ovary (Serizier and McCall, 2017). The role of Draper in apoptotic cell clearance is evolutionarily conserved. Homologues of Draper are CED-1 in *C. elegans* (Mangahas and Zhou, 2005), MEGF10 in humans and JEDI in mouse (Hamon et al., 2006). Subsequent studies implicated Draper in the phagocytosis of both *S. aureus* and *E. coli* bacteria (Cuttell et al., 2008; Hashimoto et al., 2009; Shiratsuchi et al., 2012). In particular, Hashimoto et al. identified bacterial lipoteichoic acid as a ligand for Draper in *S. aureus* engulfment by plasmatocytes (Hashimoto et al., 2009).

Another Nimrod sub-family, the Nimrod C-type (Nimrod C1-4, Eater) proteins, are transmembrane receptors containing multiple NIM domains and lacking the classical EGF repeats. Previous studies have implicated some Nimrod C-type proteins in the phagocytosis of apoptotic cells (NimC4, also called Six-Microns-Under (SIMU)) (Kurant et al., 2008) and bacteria (Eater and NimC1) (Kocks et al., 2005; Kurucz et al., 2007).

SIMU (NimC4) is highly expressed on embryonic macrophages, glia, and ectoderm. SIMU acts upstream of Draper, triggering the engulfment of apoptotic cells through PS recognition (Kurant et al., 2008; Shklyar et al., 2013). However, the detailed molecular mechanisms of interaction between SIMU and Draper remain poorly understood, since no direct interaction between the two proteins has been proven. This would suggest that other factors might be required for the SIMU/Draper-mediated embryonic efferocytosis.

So far, Eater and NimC1 represent the best characterized Nimrod-C type receptors for bacterial uptake. Eater has initially been identified by Kocks and colleagues in a genetic screen as a target of the transcription factor Serpent, which is necessary for bacterial phagocytosis (Kocks et al., 2005). Eater possesses 32 NIM repeats in its extracellular region and has a small cytosolic tail with unknown function (Kocks et al., 2005), although predicted phosphorylation sites are present. This receptor is specifically expressed on both larval and adult *Drosophila* plasmatocytes, as well as in S2 cells. Several studies using RNAi or an overlapping set of deficiencies removing *eater* and 7 flanking genes, have pointed to its crucial role in the phagocytosis of Gram-negative and Gram-positive bacteria (Horn et al., 2014; Kocks et al., 2005; Nehme et al., 2011), as well as the elimination of bacteria entering the

hemolymph by crossing the gut (Nehme et al., 2011). By using a soluble Fc-tagged receptor variant, Chung et al. reported that Eater directly binds to Gram-positive bacteria, and also to Gram-negative bacteria after Cecropin A pre-treatment. Nevertheless, the specific bacterial molecules recognized by Eater remain unknown (Chung and Kocks, 2011). Recently, an *eater* deficient mutant generated by homologous recombination confirmed the importance of Eater in the phagocytosis of Gram-positive, but not Gram-negative bacteria (Bretscher et al., 2015). Moreover, *eater* null plasmatocytes are not sessile and less adhesive (Bretscher et al., 2015). Nevertheless, how the Eater-mediated adhesion properties relate to its phagocytic ability is unknown.

NimC1 was initially identified as a target of P1, a plasmatocyte-specific antibody (Kurucz et al., 2007). NimC1 has 10 EGF-like repeats, a single transmembrane domain, and a cytosolic region with predicted phosphorylation sites. Initial RNAi studies on NimC1 provided evidence for its involvement in the phagocytosis of *S. aureus* bacteria (Kurucz et al., 2007). Moreover, immunofluorescence-based flow cytometry assays showed that NimC1 binds to bacteria *in vitro* (Zsámboki et al., 2013). A *NimC1* null mutant was recently generated by homologous recombination, revealing that NimC1 is required for the phagocytosis of latex beads and yeast zymosan particles, but is dispensable for phagocytosis of Gram-negative and Gram-positive bacteria (Melcarne et al., 2018). A role of NimC1 in the phagocytosis of latex beads is also supported by an independent study using an *in vivo* RNAi approach (Hao et al., 2018).

Use of *NimC1,eater* double mutants reveals that NimC1 does contribute to phagocytosis of both Gram-negative and Gram-positive bacteria but its function is masked by Eater. Accordingly, *NimC1,eater* double mutant is deficient in the phagocytosis of all types of bacteria. This suggests that Eater and NimC1 are the two main phagocytic receptors for bacteria in *Drosophila* and that each receptor has distinct role in microbial uptake (Melcarne et al., 2018).

3.4. Integrins

Integrins are heterodimeric proteins composed by α and β subunits, and are involved in a wide range of cellular processes, such as cell spreading and motility. In addition to their adhesion tasks, integrins were shown to mediate phagocytosis of different types of particles in several organisms. The *Drosophila* genome encodes five α- and two β-integrin subunits (Brown et al., 2000). In 2011, Nagaosa and colleagues showed the implication of βv integrin in the phagocytosis of both apoptotic cells and *S. aureus* in *Drosophila* (Nagaosa et al., 2011). Moreover, by using cell wall deficient bacteria, the authors revealed peptidoglycan as the surface determinant recognized by integrins during phagocytosis. Genetic and biochemical studies have subsequently revealed that the αPS3 and βv integrin subunits function as a heterodimer in the phagocytosis of both apoptotic cells and *S. aureus* bacteria (Nonaka et al., 2013).

3.5. Opsonins in Drosophila

Opsonins are molecules that bind to microbes and favour their engulfment by macrophages. In mammals, complement factors C3b or antibodies efficiently opsonize microbes promoting their uptake by macrophages through dedicated receptors. Little is known on the role of opsonins in microbial phagocytosis in *Drosophila*. However, the *Drosophila* genome encodes six thioester-containing proteins (Teps) structurally related to the mammalian complement factor C3 family. *Tep* genes are specifically expressed in plasmatocytes, fat body, and some barrier epithelia (Bou Aoun et al., 2011). Four of them, Tep1-Tep4, contain a signal peptide, indicating that they are secreted proteins and could function as opsonins. *Tep5* is thought to be a pseudogene (Bou Aoun et al., 2011), while *Tep6* (also called *Mcr*) plays a role in gut septate junctions (Batz et al., 2014) and is therefore unlikely to play any role in phagocytosis, although one study suggested otherwise (Stroschein-Stevenson et al., 2006). Three members of this family (Tep1, Tep2 and Tep4) show a strong upregulation after bacterial challenge, because they are regulated by the stress-responsive JAK-STAT pathway (Lagueux et al., 2000). The importance of the Tep proteins in insect phagocytosis has been first demonstrated in the mosquito *A. gambiae* (Blandin et al., 2004). A further RNAi study in *Drosophila* S2 cells (Stroschein-Stevenson et al., 2006) shed light on the function of some Tep members in binding and enhancing phagocytosis of *E. coli* (Tep2), *S. aureus* (Tep3) and *Candida albicans* (Tep6). Additional evidence in support of opsonisation in insects comes from work using a compound mutant lacking the four immune-inducible Teps (i.e. Tep1-4) (Dostálová et al., 2017). The authors found that Teps operate in both the humoral and cellular response of *Drosophila* immunity, by promoting Toll pathway activation and phagocytosis of Gram-positive bacteria (Dostálová et al., 2017). Therefore, it is likely that some Teps can bind to microbes and promote phagocytosis, as recently reported for Tep4 in *P. aeruginosa* infection (Haller et al., 2018). Despite these recent results, the specific receptors mediating the uptake of Teps remain unknown.

Another class of potential secreted opsonins are the Nimrod B type proteins, whose function remains to date poorly characterized. This subclass comprises five Nimrod B members (NimB1-B5) that contain multiple NIM domains. Close relatives of the *Drosophila NimB* genes with immune functions are found in other insect species (Estévez-Lao and Hillyer, 2014; Ju et al., 2006; Matsunaga and Fujiwara, 2002; Midega et al., 2013). Moreover, *Drosophila* NimB1 and NimB2 proteins have been shown to bind bacteria *in vitro* (Zsámboki et al., 2013). A recent study has shown that NimB5 is produced by the fat body to regulate plasmatocyte adhesion and proliferation rate. NimB5 is induced upon starvation and adjusts plasmatocyte numbers to the metabolic state of the host. Phagocytosis is however normal in *NimB5* deficient larvae (Ramond et al., submitted). Finally, evidence from the literature points to a potential role of PGRP-SC1A as a secreted opsonin for *S. aureus* bacteria and Toll pathway activation (Garver et al., 2006). Use of null mutations in the *PGRP-SC1* gene cluster did not reveal any role in Toll activation (Paredes et al., 2011). As PGRP-SC degrades peptidoglycan, it might nevertheless affect bacterial cell wall structure and consequently phagocytosis.

3.6. Down syndrome cell adhesion molecule 1 (DSCAM1)

Dscam1 is part of the Immunoglobulin (Ig) family, and possesses 95 variable exons, grouped into clusters. These clusters are in turn flanked by constant exons, leading to about 36000 potential isoforms due to alternative splicing (Armitage et al., 2012; Möller et al., 2013). Dscam1 has initially been implicated in the development of the nervous system and neuron wiring, where isoform-isoform specific interactions shape the dendritic pattern. (Wojtowicz et al., 2004; Zhan et al., 2004). In 2000, Schmucker and colleagues proved that infection leads to the production of specific secreted isoforms, with different potential "recognition abilities" that can be detected in the *Drosophila* hemolymph (Schmucker et al., 2000). However, a recent study did not reveal any change of *Dscam1* splicing upon infection (Armitage et al., 2014). Use of an RNAi targeting all *Dscam* isoforms in whole flies or plasmatocytes showed that Dscam1 is involved in binding and uptake of *E. coli* by hemocytes, and suggested that this protein might act as an opsonin (Watson et al., 2005). Moreover, *Dscam* isoforms have been implicated in the immune response of the mosquito *Anopheles gambiae* (Dong et al., 2006). Although conceptually appealing, the implication of Dscam in the fly immune response awaits further confirmation.

4. Uptake machinery and phagosome formation

Particle engulfment through phagocytosis implies an active and dynamic remodelling of the plasma membrane, which is mainly guided by the actin cytoskeleton (Fig. 2). Past studies on mammalian

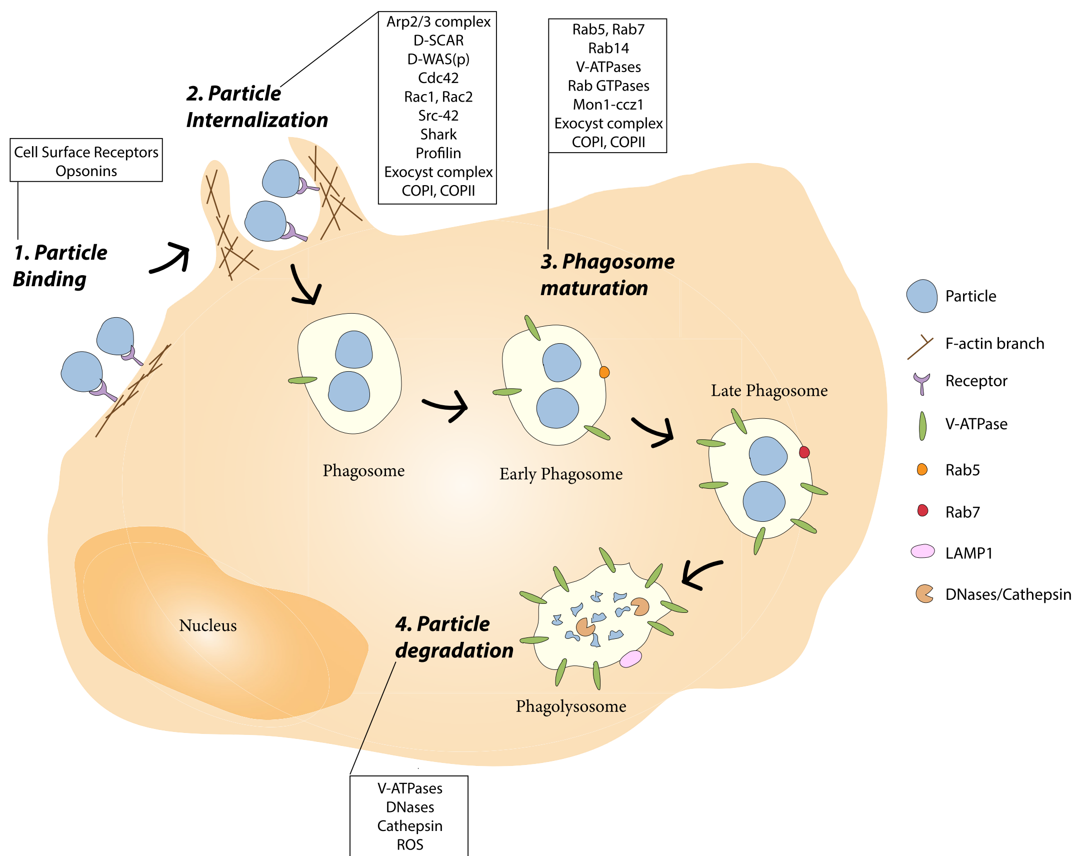

Fig. 2. The phagocytosis process in *Drosophila*.
(1–2) Recognition of the target particle by surface receptors on *Drosophila* professional phagocytes leads to F-actin branching at the engulfment site, resulting in the formation of the so-called phagocytic cup. Actin polymerization progresses around the particle, until the phagocytic protrusions fuse at the leading edges, generating a newly formed phagosome. This initial plasma membrane-derived vacuole does not have the ability to digest the internalized material. (3) Newly formed phagosomes, indeed, undergo a series of subsequent fission and fusion events (called phagosome maturation) with cellular organelles (early endosomes, late endosomes and lysosomes) (3). Rab5 is a key regulator of the initial fusion events. Another GTPase, Rab7, is needed for the late phagosome-lysosome fusion. Phagosome maturation culminates in the formation of a highly acidic phagolysosome (4). During this last step, the phagolysosome acquires important components for the final particle destruction step, such as DNAses and proteases. Boxes for each step show the main factors that have been involved in *Drosophila* phagocytosis.

phagocytosis have shown that the engulfment of a receptor-bound particle can happen via different movements of the plasma membrane: i) "zippering", typical for IgG-opsonized Fcγ-mediated phagocytosis, ii) "sinking" C3-mediated phagocytosis, or iii) "triggered" bacterial internalization by macropinocytosis (see (Flannagan et al., 2012) for a detailed review). Particle internalization by Fcγ receptor (FcγR) is the most well characterized mechanism of engulfment yet. In 2003, Pearson and colleagues proved morphological similarities between *Drosophila* plasmatocyte- and mammalian macrophage-mediated phagocytosis (Pearson et al., 2003). They observed that engulfment of *S. aureus* bacteria was primarily mediated by zippering of the plasma membrane around the microbe, suggesting that bacterial uptake occurs mainly via receptor clustering and activation. However, macropinocytosis-like and sinking (i.e. without pseudopod formation) uptake events of bacteria were also observed. Those data indicate that similar mechanisms to the mammalian C3-mediated phagocytosis exist in the fruit fly, and that *Drosophila* plasmatocytes might be able to induce bacterial engulfment without formation of a phagocytic cup. In the same study, by performing a genetic screen for hemocyte phagocytosis mutants, the authors identified various proteins required for efficient bacterial engulfment, previously known to be involved in mammalian cytoskeletal reorganization. They demonstrated that the nucleation-promoting factors D-SCAR and D-WASP are important in the engulfment of *S. aureus*. Those proteins are the *Drosophila* homologues of WAVE and WASP, which activate the Arp2/3 complex responsible for F-actin generation at the engulfment site. Other regulators of the actin network dynamics have been identified to mediate bacterial phagocytosis in *Drosophila*, such as the Rho GTPases Cdc42, Rac1, and Rac2 (which activate the nucleation factors D-SCAR and D-WASP), the Arp2/3 complex and Profilin (Agaisse et al., 2005; Avet-Rochex et al., 2005; Pearson et al., 2003; Philips et al., 2005; Stroschein-Stevenson et al., 2006; Stuart et al., 2005). Besides factors regulating membrane rearrangement, the coat-protein complex I and II (COPI, COPII) (Rämet et al., 2002; Stuart et al., 2007), as well as the exocyst complex (Stuart et al., 2007), have been shown to act as potential regulators during *Drosophila* phagocytosis.

Actin polymerization and plasma membrane remodelling around the targeted particle are tightly regulated by specific signalling pathways, which are triggered after the engagement of the engulfing receptor. Draper signalling following bacteria or apoptotic cell binding is one of the most well characterized intracellular cascades in *Drosophila* (Cuttell et al., 2008; Etchegaray et al., 2012; Hashimoto et al., 2009; Ziegenfuss et al., 2008). When glial cells phagocytose apoptotic cells, Draper induces Src42-mediated phosphorylation of the ITAM motifs located in the intracellular domain of the receptor. Next, Shark (the *Drosophila* homologue of the Syk kinase) binds via its SH2 domain to Draper's phosphorylated sites, thereby activating other downstream signals needed for apoptotic cell engulfment (Ziegenfuss et al., 2008). Draper-mediated phagocytosis of apoptotic cells is also coupled with Ca^{2+} release from the endoplasmic reticulum via the Ryanodine receptor Rya-r44F (Cuttell et al., 2008). This event leads to extracellular Ca^{2+} influx, which is regulated by the *Drosophila* junctophilin

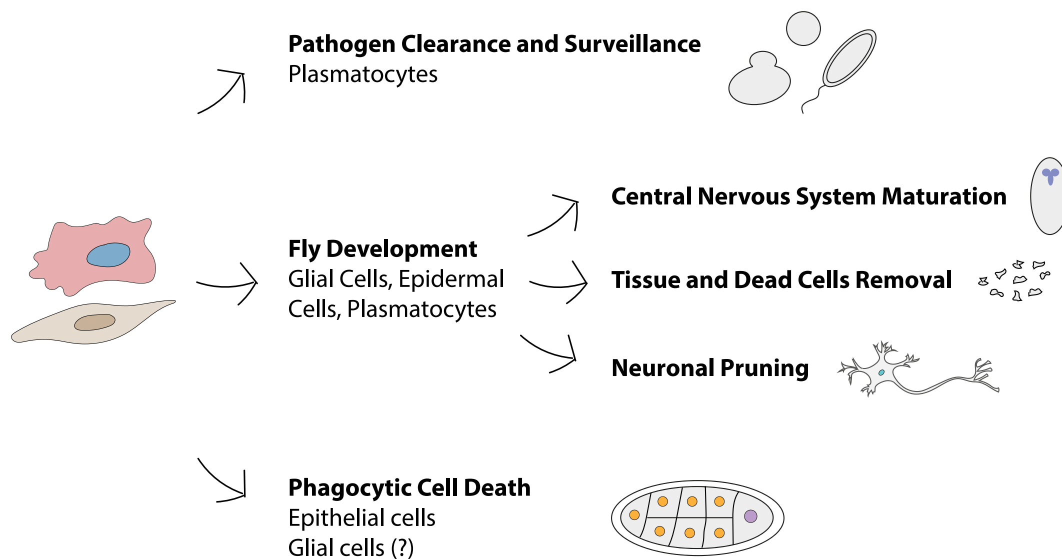

Fig. 3. Physiological roles of phagocytosis in *Drosophila*.
Professional (red cell) and non-professional (brown cell) phagocytes contribute to fly homeostasis and survival against infections. Bacterial clearance is mainly performed by the macrophage-like plasmatocytes (red). However, *Drosophila* possesses several types of non-professional phagocytes (brown) that regulate diverse aspects of fly development. During development, phagocytosis represents a key determinant for remodelling and shaping several organs. Starting at embryogenesis, mainly glia plays a critical role in CNS maturation by removing apoptotic neurons and pruned neuronal branches. Finally, recent evidence supports the existence of phagocytic cell death in the *Drosophila* ovary, where the epithelial follicle cells surrounding the egg chamber induce nurse cell death.

Undertaker, Ca^{2+} channels, and the Ca^{2+} sensor dSTIM (Cuttell et al., 2008). Interestingly, a recent study showed that Draper transcriptional levels in glia are induced by a signalling pathway downstream of Toll-6 upon binding with its ligand Spz5. As Spz5 is produced by dying neurons, this suggest a mechanism by which apoptotic cells prime glial cells to promote their efferocytosis (McLaughlin et al., 2019). Upon *S. aureus* uptake, Draper signals via Rac1 or Rac2, which are required for bacterial engulfment. Nevertheless, this study did not uncover any downstream role of Shark during Draper-mediated Gram-positive bacterial phagocytosis (Hashimoto et al., 2009).

5. Phagosome maturation and microbe destruction

Newly formed phagosomes undergo a maturation process in order to acquire bactericidal activity. The phagosome matures through fusion with endosomes and lysosomes. These organelles release their hydrolase content into the phagosome lumen (Fig. 2), a process involving small GTPases of the Rab family (Kinchen and Ravichandran, 2010; Li et al., 2009; Nieto et al., 2010). Interestingly, a comparative proteomics analysis revealed that a high percentage of phagosome-associated proteins, including Rabs, are conserved between mammals and *Drosophila* S2 cells, making these cells a suitable model for phagosome maturation studies (Stuart et al., 2007). Rab5 is generally considered a regulator of the initial fusion events, by tethering early endosomes to the newly formed phagosome (Alvarez-Dominguez et al., 1996; Duclos et al., 2000; Jahraus et al., 1998; Kinchen et al., 2008; Kitano et al., 2008; Vieira et al., 2001). Both *in vitro* and *in vivo* works on *Drosophila* confirmed the role of Rab5 in the initial steps of bacterial phagocytosis and phagosome maturation (Agaisse et al., 2005; Cheng et al., 2005; Horn et al., 2014; Peltan et al., 2012; Philips et al., 2005). As the phagosome matures, Rab5 is replaced by Rab7 in a process called Rab conversion. Different studies in worms, yeast and *Drosophila* have shown that Rab conversion might be regulated by the Rab5 effector complex Sand-1/Mon1-Ccz1 (Kinchen and Ravichandran, 2010; Poteryaev et al., 2010, 2007; Wang et al., 2002; Yousefian et al., 2013). However, a study in *Drosophila* plasmatocytes revealed that Rab7 recruitment to the phagosome might be regulated by Rab14. This GTPase localizes to both early Rab5-and late Rab7-positive *S. aureus* phagosomes, and mutant flies for *rab14* show impaired phagosome maturation (Garg and Wu, 2014). Once recruited, Rab7 is responsible for inducing phagolysosome formation. This final part of the process involves a complex of proteins named HOPS (Homotypic Fusion and Protein Sorting), which is composed of the six subunits: Vps11, Vps16, Vps18, Vps33, Vps39 and Vps41 (Akbar et al., 2011; Kinchen et al., 2008; Nickerson et al., 2009). Mutations affecting the *full-of-bacteria* (*fob*) gene, encoding the Vps16 *Drosophila* homologue, cause defects in phagosome-lysosome fusion and increase susceptibility to bacterial infection (Akbar et al., 2011; Vieira et al., 2003). A characteristic protein present on the phagolysosome membrane in mammals is Lysosomal-associated membrane protein 1 (LAMP1), which is needed for lysosome fusion with the phagosome (Garin et al., 2001; Huynh et al., 2007; Peltan et al., 2012). A *Drosophila* homologue of Lamp1 exists but has not been tested for its involvement in phagocytosis.

A key feature of phagosome maturation consists of its progressive lumen acidification, which is allowed by the activity of proton pumping vacuolar ATPase (V-ATPase). V-ATPases are found on the phagosome from the initial stages of its formation and their activity allows reaching a final pH of around 4.5–5. This final phagosome acidification is essential for proper activation of lysosomal hydrolases and consequent microbe destruction. In *Drosophila*, several V-ATPase subunits have been involved in phagosome acidification, and therefore bacterial clearance inside the phagocytes (Cheng et al., 2005; Philips et al., 2005). Proteases, notably cathepsins, are other important factors acquired through phagosome-lysosome fusion, and required for particle degradation. Cathepsin activity has been detected in *Drosophila* S2 cell phagosomes, suggesting that these cysteine proteases might be active in lysosomal compartments and participate in the fly's microbe destruction (Kocks et al., 2003). In addition, DNase II, an enzyme involved in DNA degradation, was shown to participate in bacterial clearance in phagolysosomes of adult flies (Seong et al., 2006). By using fluorescent probes, Myers et al. revealed a hemocyte reactive oxygen species response following bacterial infection. In particular, the authors could distinguish between an early ROS response, which was more pronounced in non-phagocytic cells, and a late and protracted response specific for hemocytes that have engulfed bacteria (Myers et al., 2018).

Finally, a putative glutamate transporter named Polyphemus

(Polyph) is required in plasmatocytes to successfully control bacterial growth during an infection. *polyph* mutants show altered intracellular transport of glutamate in plasmatocytes, leading to higher internal ROS, decreased phagocytosis ability, and resistance to pathogenic infection (Gonzalez et al., 2013).

6. Physiological roles of phagocytosis in *Drosophila*

Phagocytosis by *Drosophila* professional and non-professional phagocytes has critical impacts on the animal's survival, development, and homeostasis (Fig. 3). In the following paragraphs, we will describe the physiological relevance of phagocytosis during i) macrophage-mediated bacterial destruction (Shandala et al., 2013), ii) elimination of apoptotic neurons by glia (Etchegaray et al., 2016), iii) neuronal pruning by epidermal cells (Han et al., 2014), and iv) germline cell clearance by epithelial cells in the ovary (Meehan et al., 2016).

6.1. Phagocytosis of bacteria and fungi during the cellular immune response

Drosophila lives in a microbe-rich environment, and therefore needs powerful defence mechanisms to fight invading pathogens. Phagocytosis constitutes a major cellular branch of the innate immune response, which is mediated by the plasmatocytes (Figs. 3 and 4). Studies in mutant larvae and in phagocyte-depleted flies have demonstrated the importance of *Drosophila* phagocytosis in the survival against certain microbes including yeast, Gram-negative and -positive bacteria (Braun et al., 1998; Charroux and Royet, 2009; Defaye et al., 2009; Elrod-Erickson et al., 2000). In 2007, Brennan and colleagues identified a mutation called *psidin* (*phagocytes signalling impaired*) that affects a plasmatocyte lysosomal protein required to destroy internalized bacteria. Interestingly, this protein was also required for the fat body-mediated expression of Defensin upon septic injury, demonstrating that plasmatocytes contribute to Defensin expression in the fat body during both Gram-positive and Gram-negative infections (Brennan et al., 2007). Other studies found that plasmatocytes are also required for the activation of a systemic antimicrobial response in the fat body upon oral but not systemic infection (Basset et al., 2000; Charroux and Royet, 2009). It has also been proposed that plasmatocytes clear the hemolymph from the entry of intestinal microbiota or pathogens. Indeed, plasmatocyte deficient flies have higher numbers of bacteria in the hemolymph and are more susceptible to oral infection with *Serratia marcescens* (Braun et al., 1998; Nehme et al., 2007).

Plasmatocytes might rely on different recognition and signalling processes in order to efficiently clear the infection. It has been suggested that two receptors, Draper and an integrin, mediate the phagocytosis of the Gram-positive bacterium *S. aureus* in a dual recognition mechanism, by respectively recognising lipoteichoic acid and peptidoglycan (Shiratsuchi et al., 2012). Interestingly, a recent study proposed a model of bacterial phagocytosis where the Eater and NimC1 proteins function as tethering and docking receptors, respectively (Melcarne et al., 2018). The authors suggest that Eater works as the key engulfing receptor, recognising specific motifs found on the microbial surface. NimC1, on the contrary, functions in the subsequent intracellular signalling cascade, likely as a subunit of a bigger macromolecular complex.

Past studies in S2 cells allowed to identify important genes involved in the *Drosophila* cellular immune response against fungal pathogens (Levitin et al., 2007; Stroschein-Stevenson et al., 2006). An RNAi approach in S2 cells has identified specific genes required for successful recognition and uptake of *C. albicans* (Stroschein-Stevenson et al., 2006). Microarray studies allowed to identified a translational regulator, d4E-BP, as a key player in host defence against *C. albicans* (Levitin et al., 2007). d4E-BP is encoded by the *Thor* gene, which was found to be one of the most up-regulated genes in S2 cells co-incubated with *C. albicans* (Levitin et al., 2007). *Drosophila d4E-BP*^{null} flies showed increase sensitivity for *C. albicans*, but not *Saccharomyces cerevisiae* infection (Levitin et al., 2007). Moreover, the importance of phagocytosis in fly defence against fungal pathogens, has been observed upon

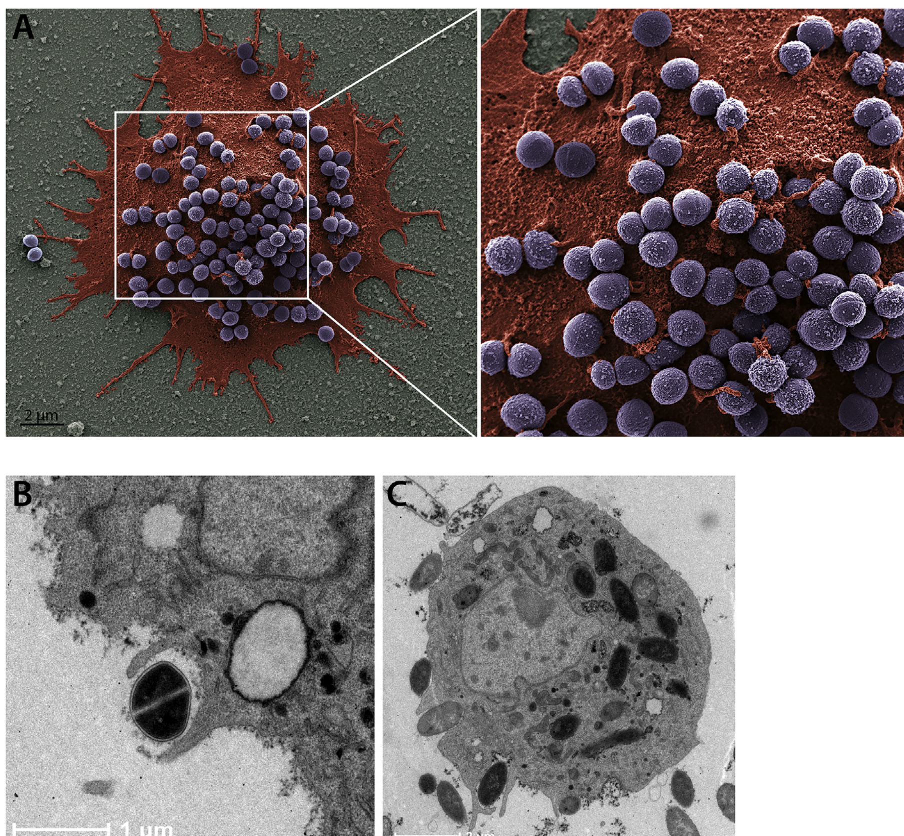

Fig. 4. Plasmatocyte-mediated bacterial phagocytosis.
(A) Plasmatocytes are *Drosophila* professional phagocytes, sharing functional features with mammalian macrophages. This scanning electron micrograph shows a plasmatocyte (red) from a third instar w^{1118} *Drosophila* larva engulfing *S. aureus* bacteria (purple). (B–C) Transmission electron micrographs of w^{1118} third instar larvae engulfing *S. aureus* (B) and *E. coli* (C) bacteria. The electron micrographs were taken in collaboration with the BioEM facility at the École Polytechnique Fédérale de Lausanne.

Zygomycetes infection (Halder et al., 2008).

Finally, it is noteworthy to mention that even though plasmatocytes have been described as the first line of defence in bacterial clearance, several pathogens have evolved strategies to escape destruction by phagocytosis. In this scenario, the phagocyte rather becomes a host cell for bacteria to proliferate in and establish the infection. *Drosophila* plasmatocytes can harbour several intracellular bacteria, in particular *M. marinum* and *L. monocytogenes* (Dionne et al., 2003; Mansfield et al., 2003). Interestingly, phagocyte-mediated sequestration of *Salmonella typhimurium* bacteria (also called "phagocytic encaspulation") was shown to act as a powerful resilience mechanism (Shinzawa et al., 2009). Phagocytic encapsulation of intracellular pathogens, which is dependent on the *Drosophila* p38 MAP kinase (Dmp38b), limits the exposure of host tissues to bacteria, and thus severe pathogenic damage. As a consequence, Dmp38b-mediated tolerance extends host survival without targeting the bacterium (Shinzawa et al., 2009).

6.2. Efferocytosis during Drosophila development

Clearance of apoptotic cells by phagocytosis is an important component of *Drosophila* development that ensures the remodelling and shaping of different organs, such as the CNS or imaginal discs. Moreover, phagocytosis of apoptotic cells by embryonic plasmatocytes is required for their maturation with regards to the tissue damage response and bacterial infection (Weavers et al., 2016).

Defects in the clearance of apoptotic cells have pathological consequences. For example, *draper* mutant flies accumulate undegraded apoptotic neurons inside glial cells throughout the lifespan, which leads to age-dependent neurodegeneration (Etchegaray et al., 2016). In the following paragraph, we describe the well-characterized maturation of the CNS via glial phagocytosis.

6.2.1. Glial phagocytosis during Drosophila nervous system maturation

The development of complex nervous systems is characterized by the generation of surplus neurons which are eliminated through apoptosis and subsequent phagocytic clearance. In parallel to the adjustment of neuronal cell number, shaping of the CNS in both vertebrates (O'Leary and Koester, 1993) and invertebrates (Tissot and Stocker, 2000) involves the removal of excessive neuronal branches, a process called pruning. In the *Drosophila* CNS, neuronal cell number is adjusted throughout the fly development at three main stages: embryogenesis, metamorphosis and emerging adult (Abrams et al., 1993; Rogulja-Ortmann et al., 2007; Tissot and Stocker, 2000; Togane et al., 2012). Glia of ectodermal origin are considered the main phagocytes responsible for shaping the CNS (Kurant et al., 2008), similar to their vertebrate counterparts, microglia and astrocytes (Fig. 5). During the late stages of *Drosophila* embryogenesis, around 30% of neurons undergo apoptosis (Abrams et al., 1993; Rogulja-Ortmann et al., 2007) and are efficiently removed by glial cells (Kurant et al., 2008). The phagocytic ability of embryonic glia to engulf and degrade apoptotic neurons is determined by the phagocytic receptors SIMU and Draper, whose genes are regulated by two transcription factors Gcm and Repo (Shklyar et al., 2014). Different studies performed in *simu* and *draper* mutant embryos have demonstrated that SIMU is required for recognition and engulfment of apoptotic neurons by glia, whereas Draper is mostly needed for their degradation (Freeman et al., 2003; Kurant et al., 2008; Shklyar et al., 2014). It has been shown that the *Drosophila* JNK pathway in glial cells can enhance clearance of apoptotic neurons, by promoting their degradation without interfering with the expression of SIMU and Draper (Shklover et al., 2015b). During metamorphosis, CNS development is characterized by two different types of neuronal efferocytosis: phagocytosis of apoptotic neurons and neuronal pruning. Removal of apoptotic neurons in pupae takes place during the first quarter of metamorphosis, and it is also mediated by glial cells (Hilu-Dadia et al., 2018; Tasdemir-Yilmaz and Freeman, 2014). Professional phagocytes seem not to be involved in efferocytosis in the CNS at this developmental stage (Cantera and Technau, 1996; Hilu-Dadia et al., 2018; Tasdemir-Yilmaz and Freeman, 2014). Interestingly, recent work has shown that Draper is required for both engulfment and degradation of dying neurons by glia (Hilu-Dadia et al., 2018; Tasdemir-Yilmaz and Freeman, 2014). On the contrary, SIMU, although highly expressed at this developmental stage, is not present in glia but rather on macrophages outside the CNS. The authors suggest that Draper might activate two distinct signalling pathways to trigger removal of apoptotic neurons. One is the JNK pathway in astrocytes and in ensheathing glia, while the other is still unknown (Hilu-Dadia et al., 2018). Pruning of larval axons during metamorphosis is mediated by the glial receptor Draper and Ced-6, and is not caspase dependent (Awasaki et al., 2006). Interestingly, the more recent studies demonstrate that astrocytes use distinct molecular programs to engulf neuronal debris originated from neuronal death or local pruning (Hilu-Dadia et al., 2018; Tasdemir-Yilmaz and Freeman, 2014). The last phase of neuronal elimination takes place in emerging flies (Peterson et al., 2002). However, the cells responsible for neuron removal are still unknown. A mechanism proposed by Kato and collaborators suggested that dying neurons are able to induce proliferation of the surrounding glia, which eventually participate in their engulfment (Kato et al., 2009). Flies deficient for the two main receptors for apoptotic cells, *draper* and *simu*, are however viable but their lifespan is reduced and their CNS shows accumulation of apoptotic bodies.

6.2.2. Peripheral dendrite clearance by epidermal cells

In *Drosophila*, peripheral neurons undergo dendrite and axon pruning as part of developmental apoptosis during metamorphosis. In this context, the degenerating dendrites are efficiently removed by epidermal cells, rather than by professional phagocytes or glia, even though plasmatocytes are found in contact with those neurons (Han et al., 2014). Recognition of the degenerating dendrites by epidermal cells is mediated by the engulfing receptor Draper. Interestingly, loss of function of *crq* in epidermal cells does not affect dendrite uptake, but

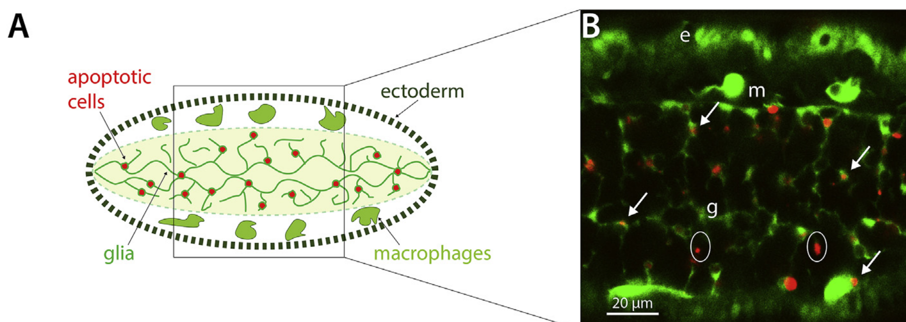

Fig. 5. Phagocytic cell populations in the late *Drosophila* embryo.
(A) Schematic ventral view of stage 16 embryo, showing that at this developmental stage apoptosis is largely restricted to the CNS (thin dashed oval). Large dashed oval depicts ectodermal cells, red dots represent AnnexinV-labelled apoptotic cells (inside or outside green-labelled phagocytes), glial cells are depicted as green network inside CNS, macrophages are shown as big green cells outside CNS, showing that they do not enter the CNS at this stage. (B) Confocal snapshot of stage 16 live embryo at the area depicted as black frame in A. Ectoderm (e), glia (g) and macrophages (m) are labelled with a *simu-cytGFP* transgenic construct. Apoptotic cells are labelled by injected fluorescent AnnexinV (red). Most of AnnexinV-positive cells are found inside phagocytic glia and macrophages (arrows). Circles highlight unengulfed AnnexinV-labelled cells.

Table 1
Phagocytic receptors and opsonins in *Drosophila*.

Name	Phagocytic function	References
Drosophila Scavenger receptor C1	Gram-positive	Pearson et al. (1995)
	Gram-negative	Ramët et al. (2001)
Croquemort	Apoptotic cells	Franc et al. (1996)
	Gram-positive (*S. aureus*)	Stuart et al. (2005)
Peste	*Mycobacterium fortuitum*	Philips et al. (2005)
	Listeria monocytogenes	Agaisse et al. (2005)
PGRP-LC	Gram-negative	Ramët et al. (2002)
Draper	Apoptotic cells	Freeman et al. (2003)
	Gram-positive	Cuttel et al., (2008), Hashimoto et al., (2009),
	Gram-negative	Shiratsuchi et al. (2012)
Eater	Gram-positive	Kocks et al., (2005),
	Gram-negative	Bretscher et al. (2015)
NimC1	Latex beads	Kurucz et al. (2007)
	Zymosan particles	Melcarne et al. (2018)
		Hao et al. (2018)
Integrin	Gram-positive	Shiratsuchi et al. (2012)
	Apoptotic cells	Nagaosa et al. (2011)
NimC4/SIMU	Apoptotic cells	Kurant et al. (2008)
DSCAM1	Gram-negative (*E. coli*)	Watson et al. (2005)
TEP2	Gram-negative (*E. coli*)	Stroschein-Stevenson et al. (2006)
TEP3	Gram-positive (*S. aureus*)	Stroschein-Stevenson et al. (2006)
TEP6	Yeast (*C. albicans*)	Stroschein-Stevenson et al. (2006)
TEP1-4	Gram-positive	Dostalova et al. (2017)
Nimrod B	Potential opsonins	
PGRP-SC1	Gram-positive (*S. aureus*)	Garver et al. (2006)

rather phagosome maturation. The authors also identified an additional CD36 family protein, Debris Buster, required for late stages of phagosome maturation (Han et al., 2014).

6.2.3. Phagocytosis mediated-cell death in Drosophila, an insight from the fly ovary

Since its discovery, phagocytosis has always been thought to be a beneficial mechanism to remove unwanted cells or infecting microbes. However, recent advances in the phagocytosis field have shed light on specific mechanisms whereby living cells can be taken up by phagocytes. One of those processes has been named “phagoptosis”, or “primary phagocytosis” (Brown and Neher, 2012). Phagoptosis has been defined as phagocyte mediated cell death, where phagocytes induce death of living cells by mediating their engulfment. It has been suggested that phagoptosis is used among several multicellular organisms to mediate both homeostatic functions and cellular stress responses (Brown and Neher, 2012). However, if not tightly regulated, phagoptosis can lead to pathological states. A well characterized mechanism of mammalian phagoptosis is given by microglial engulfment of viable neurons, leading to their “killing” and consequent neurodegenerative conditions. In *Drosophila*, phagocytic receptor expression in glia must be tightly regulated, as expression of SIMU in adult glia (where it is not normally expressed) or overexpression of Draper triggers neuronal degeneration by phagoptosis of living cells (E. Kurant, personal communication). This study reveals that dysregulation of phagocytic receptors in glia can promote neurodegeneration by inducing neuronal loss.

Recent studies on the *Drosophila* ovary have given a first hint of the presence of phagoptosis in that organ (Etchegaray et al., 2012; Timmons et al., 2016). The *Drosophila* ovary represents a well-established system for studying efferocytosis mediated by non-professional neighbouring cells, since no circulating macrophages are present in that organ. The egg chamber is the single unit of the ovary, and it consists of three main cell types: nurse cells, oocyte and follicle cells. While nurse cells and oocyte are germline-derived, follicle cells are somatic epithelial cells that form a protective barrier around the egg chamber. Late oogenesis in *Drosophila* is characterized by a developmental form of non-apoptotic death of the nurse cells. In this physiological context, Timmons and colleagues showed that the phagocytic machinery of the follicle cells is required to induce the death of the nurse cells, as well as their subsequent removal (Timmons et al., 2016). As a further proof of this mechanism, inhibition of the phagocytosis genes in follicle cells impaired germline death and clearance. These findings indicate that phagoptosis might occur in *Drosophila* as a physiological mechanism of developmental cell death.

7. Conclusions

Phagocytosis represents a complex and vital mechanism in the life of multicellular organisms. 100 years after its discovery, we have gained an enormous knowledge of its underlying molecular mechanisms, and its diverse implications in development and tissue homeostasis. The use of modern molecular biology tools and simple but powerful tractable model organisms such as *Drosophila* has contributed significantly to our recent advances in the phagocytosis field. Since 2001, many phagocytic transmembrane receptors, potential opsonins, and in some cases their ligands, have been discovered (Table 1). Among them, the Nimrod family, which contains key phagocytic receptors and potential opsonins, deserves special interest as accumulating genetic and biochemical evidence points to their critical role in phagocytosis of both bacteria and apoptotic cells. Future research should narrow down at which step these receptors are working, how they collaborate between each other, and the downstream components mediating the engulfment process. Past RNAi screens and proteomic studies have strengthened our knowledge on the molecular mechanisms underlying both particle uptake and phagosome maturation. Nevertheless, the *Drosophila* signalling processes that connect particle recognition with downstream engulfment events are globally poorly understood. For instance, the mechanisms that contribute to microbe or apoptotic cell digestion are barely defined. How phagocytosis is coupled to plasmatocyte or glia metabolism is an interesting future line of research. Are ingested microbes fuelling the metabolism of plasmatocytes or rather evacuated as waste? Understanding how phagocytic ability develops during differentiation should provide insight into what makes a cell competent for phagocytosis. For instance, is the expression of phagocytic receptors sufficient to turn a cell into a professional phagocyte? How should the level of phagocytic receptors be adjusted to prevent phagoptosis while efficiently removing apoptotic cells? Integrating phagocytosis with physiology, in both normal and pathological contexts, is likely to be important. Hemocytes have recently been implicated in *Drosophila* models of cancer, neurodegeneration and aging, and it would be insightful to understand how phagocytosis *per se* contributes to these conditions.

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References

Abrams, J.M., White, K., Fessler, L.I., Steller, H., 1993. Programmed cell death during Drosophila embryogenesis. Development 117, 29–43.

Agaisse, H., Burrack, L.S., Philips, J., Rubin, E.J., Perrimon, N., Higgins, D.E., 2005. Genome-wide RNAi screen for host factors required for intracellular bacterial infection. Science 1248, 1116008. https://doi.org/10.1126/science.1116008.

Akbar, M.A., Tracy, C., Kahr, W.H.A., Krämer, H., 2011. Gene is required for phagosome maturation during immune defense in. https://doi.org/10.1083/jcb.201008119.

Alvarez-Dominguez, C., Barbieri, A.M., Berón, W., Wandinger-Ness, A., Stahl, P.D., 1996. Phagocytosed live Listeria monocytogenes influences rab5-regulated in vitro phagosome-endosome fusion. J. Biol. Chem. 271, 13834–13843. https://doi.org/10.1074/jbc.271.23.13834.

Armitage, S.A.O., Freiburg, R.Y., Kurtz, J., Bravo, I.G., 2012. The evolution of Dscam genes across the arthropods. BMC Evol. Biol. 12, 1–15. https://doi.org/10.1186/1471-2148-12-53.

Armitage, S.A.O., Sun, W., You, X., Kurtz, J., Schmucker, D., Chen, W., 2014. Quantitative Profiling of Drosophila melanogaster Dscam1 isoforms reveals no changes in splicing after bacterial exposure. PLoS One 9. https://doi.org/10.1371/journal.pone.0108660.

Avet-Rochex, A., Bergeret, E., Attree, I., Meister, M., Fauvarque, M.O., 2005. Suppression of Drosophila cellular immunity by directed expression of the ExoS toxin GAP domain of Pseudomonas aeruginosa. Cell Microbiol. 7, 799–810. https://doi.org/10.1111/j.1462-5822.2005.00512.x.

Awasaki, T., Tatsumi, R., Takahashi, K., Arai, K., Nakanishi, Y., Ueda, R., Ito, K., 2006. Essential role of the apoptotic cell engulfment genes draper and ced-6 in programmed axon pruning during Drosophila metamorphosis. Neuron 50, 855–867. https://doi.org/10.1016/j.neuron.2006.04.027.

Basset, A., Khush, R.S., Braun, A., Gardan, L., Boccard, F., Hoffmann, J.A., Lemaitre, B., 2000. The phytopathogenic bacteria Erwinia carotovora infects Drosophila and activates an immune response. Proc. Natl. Acad. Sci. Unit. States Am. 97, 3376–3381. https://doi.org/10.1073/pnas.97.7.3376.

Batz, T., Forster, D., Luschnig, S., 2014. The transmembrane protein Macroglobulin complement-related is essential for septate junction formation and epithelial barrier function in Drosophila. Development 141, 899–908. https://doi.org/10.1242/dev.102160.

Blandin, S., Shiao, S.H., Moita, L.F., Janse, C.J., Waters, A.P., Kafatos, F.C., Levashina, E.A., 2004. Complement-like protein TEP1 is a determinant of vectorial capacity in the malaria vector Anopheles gambiae. Cell 116, 661–670. https://doi.org/10.1016/S0092-8674(04)00173-4.

Bork, P., Downing, A.K., Kieffer, B., Campbell, I.D., 1996. Structure and distribution of modules in extracellular proteins. Q. Rev. Biophys. 29, 119. https://doi.org/10.1017/S0033583500005783.

Bou Aoun, R., Hetru, C., Troxler, L., Doucet, D., Ferrandon, D., Matt, N., 2011. Analysis of thioester-containing proteins during the innate immune response of Drosophila melanogaster. J. Innate Immun. 3, 52–64. https://doi.org/10.1159/000321554.

Braun, A., Hoffmann, J.A., Meister, M., 1998. Analysis of the Drosophila host defense in domino mutant larvae, which are devoid of hemocytes. Proc. Natl. Acad. Sci. Unit. States Am. 95, 14337–14342. https://doi.org/10.1073/pnas.95.24.14337.

Brennan, C.A., Delaney, J.R., Schneider, D.S., Anderson, K.V., 2007. Psidin is required in Drosophila blood cells for both phagocytic degradation and immune activation of the fat body. Curr. Biol. 17, 67–72. https://doi.org/10.1016/j.cub.2006.11.026.

Bretscher, A.J., Honti, V., Binggeli, O., Burri, O., Poidevin, M., Kurucz, E., Zsamboki, J., Ando, I., Lemaitre, B., 2015. The Nimrod transmembrane receptor Eater is required for hemocyte attachment to the sessile compartment in Drosophila melanogaster. Biol. Open 4, 355–363. https://doi.org/10.1242/bio.201410595.

Brown, G.C., Neher, J.J., 2012. Eaten alive! Cell death by primary phagocytosis: "Phagoptosis. Trends Biochem. Sci. 37, 325–332. https://doi.org/10.1016/j.tibs.2012.05.002.

Brown, M.S., Goldstein, J.L., 1983. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. Annu. Rev. Biochem. 52, 223–261. https://doi.org/10.1146/annurev.bi.52.070183.001255.

Brown, N.H., Gregory, S.L., Martin-Bermudo, M.D., 2000. Integrins as mediators of morphogenesis in Drosophila. Dev. Biol. 223, 1–16. https://doi.org/10.1006/dbio.2000.9711.

Callebaut, I., Mignotte, V., Souchet, M., Mornon, J.P., 2003. EMI domains are widespread and reveal the probable orthologs of the Caenorhabditis elegans CED-1 protein. Biochem. Biophys. Res. Commun. 300, 619–623. https://doi.org/10.1016/S0006-291X(02)02904-2.

Cantera, R., Technau, G.M., 1996. Glial cells phagocytose neuronal debris during the metamorphosis of the central nervous system in Drosophila melanogaster. Dev. Gene. Evol. 277–280. https://doi.org/10.1007/s004270050052.

Charroux, B., Royet, J., 2009. Elimination of plasmatocytes by targeted apoptosis reveals their role in multiple aspects of the Drosophila immune response. Proc. Natl. Acad. Sci. Unit. States Am. 106, 9797–9802. https://doi.org/10.1073/pnas.0903971106.

Cheng, L.W., Viala, J.P.M., Stuurman, N., Wiedemann, U., Vale, R.D., Portnoy, D.A., 2005. Use of RNA interference in Drosophila S2 cells to identify host pathways controlling compartmentalization of an intracellular pathogen. Proc. Natl. Acad. Sci. U. S. A 102, 13646–13651. https://doi.org/10.1073/pnas.0506461102.

Chung, Y.S.A., Kocks, C., 2011. Recognition of pathogenic microbes by the Drosophila phagocytic pattern recognition receptor eater. J. Biol. Chem. 286, 26524–26532. https://doi.org/10.1074/jbc.M110.214007.

Cosson, P., Soldati, T., 2008. Eat, kill or die: when amoeba meets bacteria. Curr. Opin. Microbiol. 11, 271–276. https://doi.org/10.1016/j.mib.2008.05.005.

Cuttell, L., Vaughan, A., Silva, E., Escaron, C.J., Lavine, M., Van Goethem, E., Eid, J.P., Quirin, M., Franc, N.C., 2008. Undertaker, a Drosophila junctophilin, links draper-mediated phagocytosis and calcium homeostasis. Cell 135, 524–534. https://doi.org/10.1016/j.cell.2008.08.033.

Defaye, A., Evans, I., Crozatier, M., Wood, W., Lemaitre, B., Leulier, F., 2009. Genetic ablation of Drosophila phagocytes reveals their contribution to both development and resistance to bacterial infection. J. Innate Immun. 1, 322–334. https://doi.org/10.1159/000210264.

Dionne, M.S., Ghori, N., Schneider, D.S., 2003. Drosophila melanogaster is a genetically tractable model host for Mycobacterium marinum. Infect. Immun. 71, 3540–3550. https://doi.org/10.1128/IAI.71.6.3540-3550.2003.

Dong, Y., Taylor, H.E., Dimopoulos, G., 2006. AgDscam, a hypervariable immunoglobulin domain-containing receptor of the Anopheles gambiae innate immune system. PLoS Biol. 4, 1137–1146. https://doi.org/10.1371/journal.pbio.0040229.

Dostálová, A., Rommelaere, S., Poidevin, M., Lemaitre, B., 2017. Thioester-containing proteins regulate the Toll pathway and play a role in Drosophila defence against microbial pathogens and parasitoid wasps. BMC Biol. 15, 1–16. https://doi.org/10.1186/s12915-017-0408-0.

Duclos, S., Diez, R., Garin, J., Papadopoulou, B., Descoteaux, A., Stenmark, H., Desjardins, M., 2000. Rab5 Regulates the Kiss and Run Fusionbetween Phagosomes and Endosomes and the Acquisition of Phagosome Leishmanicidial Properties in RAW 264.7 Macrophages. pp. 3531–3541.

Elrod-Erickson, S., Schneider, D., Mishr, M., 2000. Interactions between the cellular and humoral immune responses in Drosophila. Curr. Biol. 10, 781–784.

Estévez-Lao, T.Y., Hillyer, J.F., 2014. Involvement of the Anopheles gambiae Nimrod gene family in mosquito immune responses. Insect Biochem. Mol. Biol. 44, 12–22. https://doi.org/10.1016/j.ibmb.2013.10.008.

Etchegaray, J.I., Elguero, E.J., Tran, J.A., Sinatra, V., Feany, M.B., McCall, K., 2016. Defective phagocytic corpse processing results in neurodegeneration and can Be rescued by TORC1 activation. J. Neurosci. 36, 3170–3183. https://doi.org/10.1523/JNEUROSCI.1912-15.2016.

Etchegaray, J.I., Timmons, A.K., Klein, A.P., Pritchett, T.L., Welch, E., Meehan, T.L., Li, C., McCall, K., 2012. Draper acts through the JNK pathway to control synchronous engulfment of dying germline cells by follicular epithelial cells. Development 139, 4029–4039. https://doi.org/10.1242/dev.082776.

Flannagan, R.S., Jaumouillé, V., Grinstein, S., 2012. The cell biology of phagocytosis. Annu. Rev. Pathol. 7, 61–98. https://doi.org/10.1146/annurev-pathol-011811-132445.

Franc, N.C., Dimarcq, J.L., Lagueux, M., Hoffmann, J., Ezekowitz, R.A.B., 1996. Croquemort, a novel drosophila hemocyte/macrophage receptor that recognizes apoptotic cells. Immunity 4, 431–443. https://doi.org/10.1016/S1074-7613(00)80410-0.

Franc, N.C., Heitzler, P., Ezekowitz, R. a, White, K., 1999. Requirement for croquemort in phagocytosis of apoptotic cells in Drosophila. Science 284, 1991–1994. https://doi.org/7588.

Freeman, M.R., Delrow, J., Kim, J., Johnson, E., Doe, C.Q., 2003. Unwrapping glial biology: Gcm target genes regulating glial development, diversification, and function. Neuron 38, 567–580. https://doi.org/10.1016/S0896-6273(03)00289-7.

Garg, A., Wu, L.P., 2014. Drosophila Rab14 mediates phagocytosis in the immune response to staphylococcus aureus. Cell Microbiol. 16, 296–310. https://doi.org/10.1111/cmi.12220.

Garin, J., Diez, R., Kieffer, S., Dermine, J.-F., Duclos, S., Gagnon, E., Sadoul, R., Rondeau, C., Desjardins, M., 2001. The phagosome proteome. J. Cell Biol. 152, 165–180. https://doi.org/10.1083/jcb.152.1.165.

Garver, L.S., Wu, J., Wu, L.P., 2006. The peptidoglycan recognition protein PGRP-SC1a is essential for Toll signaling and phagocytosis of Staphylococcus aureus in Drosophila. Proc. Natl. Acad. Sci. Unit. States Am. 103, 660–665. https://doi.org/10.1073/pnas.0506182103.

Gonzalez, E.A., Garg, A., Tang, J., Nazario-Toole, A.E., Wu, L.P., 2013. A glutamate-dependent redox system in blood cells is integral for phagocytosis in Drosophila melanogaster. Curr. Biol. 23, 2319–2324. https://doi.org/10.1016/j.cub.2013.09.061.

Guillou, A., Troha, K., Wang, H., Franc, N.C., Buchon, N., 2016. The Drosophila CD36 homologue croquemort is required to maintain immune and gut homeostasis during development and aging. PLoS Pathog. 12, 1–27. https://doi.org/10.1371/journal.ppat.1005961.

Halder, G., Hu, J., Gilliet, M., Xiao, L., Zal, T., Kontoyiannis, D.P., Chamilos, G., Lewis, R.E., 2008. Drosophila melanogaster as a model host to dissect the immunopathogenesis of zygomycosis. Proc. Natl. Acad. Sci. Unit. States Am. 105, 9367–9372. https://doi.org/10.1073/pnas.0709578105.

Haller, S., Franchet, A., Hakkim, A., Chen, J., Drenkard, E., Yu, S., Schirmeier, S., Li, Z., Martins, N., Ausubel, F.M., Liégeois, S., Ferrandon, D., 2018. Quorum-sensing regulator RhlR but not its autoinducer RhlI enables *Pseudomonas* to evade opsonization. EMBO Rep., e44880. https://doi.org/10.15252/embr.201744880.

Hamon, M., Bierne, H., Cossart, P., 2006. Listeria monocytogenes: a multifaceted model. Nat. Rev. Microbiol. 4, 423–434. https://doi.org/10.1038/nrmicro1413.

Han, C., Song, Y., Xiao, H., Wang, D., Franc, N.C., Yeh Jan, L., Jan, Y.N., 2014. Epidermal cells are the primary phagocytes in the fragmentation and clearance of degenerating dendrites in Drosophila. Neuron 81, 544–560. https://doi.org/10.1016/j.neuron.2013.11.021.

Hao, Y., Yu, S., Luo, F., Jin, L.H., 2018. Jumu is required for circulating hemocyte differentiation and phagocytosis in Drosophila. 3, 1–20.

Hashimoto, Y., Tabuchi, Y., Sakurai, K., Kutsuna, M., Kurokawa, K., Awasaki, T., Sekimizu, K., Nakanishi, Y., Shiratsuchi, A., 2009. Identification of lipoteichoic acid as a ligand for draper in the phagocytosis of Staphylococcus aureus by Drosophila hemocytes. J. Immunol. 183, 7451–7460. https://doi.org/10.4049/jimmunol.0901032.

Herboso, L., Talamillo, A., Pérez, C., Barrio, R., 2011. Expression of the scavenger receptor class B type I (SR-BI) family in drosophila melanogaster. Int. J. Dev. Biol. 55, 603–611. https://doi.org/10.1387/ijdb.103254lh.

Hilu-Dadia, R., Hakim-Mishnaevski, K., Levy-Adam, F., Kurant, E., 2018. Draper-mediated JNK signaling is required for glial phagocytosis of apoptotic neurons during Drosophila metamorphosis. Glia 66, 1520–1532. https://doi.org/10.1002/glia.23322.

Horn, L., Leips, J., Starz-Gaiano, M., 2014. Phagocytic ability declines with age in adult Drosophila hemocytes. Aging Cell 13, 719–728. https://doi.org/10.1111/acel.12227.

Huynh, K.K., Eskelinen, E.L., Scott, C.C., Malevanets, A., Saftig, P., Grinstein, S., 2007. LAMP proteins are required for fusion of lysosomes with phagosomes. EMBO J. 26,

313–324. https://doi.org/10.1038/sj.emboj.7601511.
Irving, P., Ubeda, J.M., Doucet, D., Troxler, L., Lagueux, M., Zachary, D., Hoffmann, J.A., Hetru, C., Meister, M., 2005. New insights into Drosophila larval haemocyte functions through genome-wide analysis. Cell Microbiol. 7, 335–350. https://doi.org/10.1111/j.1462-5822.2004.00462.x.
Jahraus, A., Tjelle, T.E., Berg, T., Habermann, A., Storrie, B., Ullrich, O., Griffiths, G., 1998. In vitro fusion of phagosomes with different endocytic organelles from J774 macrophages. J. Biol. Chem. 273, 30379–30390. https://doi.org/10.1074/jbc.273.46.30379.
Ju, J.S., Cho, M.H., Brade, L., Kim, J.H., Park, J.W., Ha, N.-C., Soderhall, I., Soderhall, K., Brade, H., Lee, B.L., 2006. A novel 40-kDa protein containing six repeats of an epidermal growth factor-like domain functions as a pattern recognition protein for lipopolysaccharide. J. Immunol. 177, 1838–1845. https://doi.org/10.4049/jimmunol.177.3.1838.
Kato, K., Awasaki, T., Ito, K., 2009. Neuronal programmed cell death induces glial cell division in the adult Drosophila brain. Development 136, 51–59. https://doi.org/10.1242/dev.023366.
Kinchen, J.M., Doukoumetzidis, K., Almendinger, J., Stergiou, L., Tosello-Trampont, A., Sifri, C.D., Hengartner, M.O., Ravichandran, K.S., 2008. A novel pathway for phagosome maturation during engulfment of apoptotic cells. Nat. Cell Biol. 556–566. https://doi.org/10.1038/ncb1718.A.
Kinchen, J.M., Ravichandran, K.S., 2010. Identification of two evolutionarily conserved genes regulating processing of engulfed apoptotic cells. Nature 464, 778–782. https://doi.org/10.1038/nature08853.
Kitano, M., Nakaya, M., Nakamura, T., Nagata, S., Matsuda, M., 2008. Imaging of Rab5 activity identifies essential regulators for phagosome maturation. Nature 453, 241–245. https://doi.org/10.1038/nature06857.
Kocks, C., Cho, J.H., Nehme, N., Ulvila, J., Pearson, A.M., Meister, M., Strom, C., Conto, S.L., Hetru, C., Stuart, L.M., Stehle, T., Hoffmann, J.A., Reichhart, J.M., Ferrandon, D., Rämet, M., Ezekowitz, R.A.B., 2005. Eater, a transmembrane protein mediating phagocytosis of bacterial pathogens in Drosophila. Cell 123, 335–346. https://doi.org/10.1016/j.cell.2005.08.034.
Kocks, C., Maehr, R., Overkleeft, H.S., Wang, E.W., Iyer, L.K., Lennon-Duménil, A.-M., Ploegh, H.L., Kessler, B.M., 2003. Functional proteomics of the active cysteine protease content in *Drosophila* S2 cells. Mol. Cell. Proteomics 2, 1188–1197. https://doi.org/10.1074/mcp.M300067-MCP200.
Kuraishi, T., Nakagawa, Y., Nagaosa, K., Hashimoto, Y., Ishimoto, T., Moki, T., Fujita, Y., Nakayama, H., Dohmae, N., Shiratsuchi, A., Yamamoto, N., Ueda, K., Yamaguchi, M., Awasaki, T., Nakanishi, Y., 2009. Pretaporter, a Drosophila protein serving as a ligand for Draper in the phagocytosis of apoptotic cells. EMBO J. 28, 3868–3878. https://doi.org/10.1038/emboj.2009.343.
Kurant, E., Axelrod, S., Leaman, D., Gaul, U., 2008. Six-microns-under acts upstream of draper in the glial phagocytosis of apoptotic neurons. Cell 133, 498–509. https://doi.org/10.1016/j.cell.2008.02.052.
Kurucz, É., Márkus, R., Zsámboki, J., Folkl-Medzihradszky, K., Darula, Z., Vilmos, P., Udvardy, A., Krausz, I., Lukacsovich, T., Gateff, E., Zettervall, C.J., Hultmark, D., Andó, I., 2007. Nimrod, a putative phagocytosis receptor with EGF repeats in Drosophila plasmatocytes. Curr. Biol. 17, 649–654. https://doi.org/10.1016/j.cub.2007.02.041.
Lagueux, M., Perrodou, E., Levashina, E.A., Capovilla, M., Hoffmann, J.A., 2000. Constitutive expression of a complement-like protein in Toll and JAK gain-of-function mutants of Drosophila. Proc. Natl. Acad. Sci. Unit. States Am. 97, 11427–11432. https://doi.org/10.1073/pnas.97.21.11427.
Lazzaro, B.P., 2005. Elevated polymorphism and divergence in the class C scavenger receptors of Drosophila melanogaster and D. simulans. Genetics 169, 2023–2034. https://doi.org/10.1534/genetics.104.034249.
Lemaitre, B., Hoffmann, J., 2007. The host defense of *Drosophila melanogaster*. Annu. Rev. Immunol. 25, 697–743. https://doi.org/10.1146/annurev.immunol.25.022106.141615.
Levitin, A., Marcil, A., Tettweiler, G., Laforest, M.J., Oberholzer, U., Alarco, A.M., Thomas, D.Y., Lasko, P., Whiteway, M., 2007. Drosophila melanogaster Thor and response to Candida albicans infection. Eukaryot. Cell 6, 658–663. https://doi.org/10.1128/EC.00346-06.
Li, W., Zou, W., Zhao, D., Yan, J., Zhu, Z., Lu, J., Wang, X., 2009. C. Elegans Rab GTPase Activating Protein TBC-2 Promotes Cell Corpse Degradation by Regulating the Small GTPase RAB-5 2455. pp. 2445–2455. https://doi.org/10.1242/dev.035949.
Logan, M.A., Hackett, R., Doherty, J., Sheehan, A., Speese, S.D., Freeman, M.R., 2012. Negative regulation of glial engulfment activity by Draper terminates glial responses to axon injury. Nat. Neurosci. 15, 722–730. https://doi.org/10.1038/nn.3066.
Manaka, J., Kuraishi, T., Shiratsuchi, A., Nakai, Y., Higashida, H., Henson, P., Nakanishi, Y., 2004. Draper-mediated and phosphatidylserine-independent phagocytosis of apoptotic cells by Drosophila hemocytes/macrophages. J. Biol. Chem. 279, 48466–48476. https://doi.org/10.1074/jbc.M408597200.
Mangahas, P.M., Zhou, Z., 2005. Clearance of apoptotic cells in Caenorhabditis elegans. Semin. Cell Dev. Biol. 16, 295–306. https://doi.org/10.1016/j.semcdb.2004.12.005.
Mansfield, B.E., Dionne, M.S., Schneider, D.S., Freitag, N.E., 2003. Exploration of host-pathogen interactions using Listeria monocytogenes and Drosophila melanogaster. Cell Microbiol. 5, 901–911. https://doi.org/10.1046/j.1462-5822.2003.00329.x.
Martinek, N., Shahab, J., Saathoff, M., Ringuette, M., 2008. Haemocyte-derived SPARC is required for collagen-IV-dependent stability of basal laminae in Drosophila embryos. J. Cell Sci. 124 670–670. https://doi.org/10.1242/jcs.086819.
Matsunaga, T.M., Fujiwara, H., 2002. Identification and characterization of genes abnormally expressed in wing-deficient mutant (flugellos) of the silkworm, Bombyx mori. Insect Biochem. 32, 691–699.
McLaughlin, C.N., Perry-Richardson, J.J., Coutinho-Budd, J.C., Broihier, H.T., 2019. Dying neurons utilize innate immune signaling to prime glia for phagocytosis during development. Dev. Cell 0, 506–522. e6. https://doi.org/10.1016/j.devcel.2018.12.019.
Meehan, T.L., Joudi, T.F., Timmons, A.K., Taylor, J.D., Habib, C.S., Peterson, J.S., Emmanuel, S., Franc, N.C., McCall, K., 2016. Components of the engulfment machinery have distinct roles in corpse processing. PLoS One 11, 1–22. https://doi.org/10.1371/journal.pone.0158217.
Melcarne, C., Ramond, E., Dudzic, J.P., Bretscher, A.J., Kurucz, É., Andó, I., Lemaitre, B., 2018. Two Nimrod Receptors, NimC1 and Eater, Synergistically Contribute to Bacterial Phagocytosis in. https://doi.org/10.1101/479550.
Mellroth, P., Karlsson, J., Steiner, H., 2003. A scavenger function for a Drosophila peptidoglycan recognition protein. J. Biol. Chem. 278, 7059–7064. https://doi.org/10.1074/jbc.M208900200.
Midega, J., Blight, J., Lombardo, F., Povelones, M., Kafatos, F., Christophides, G.K., 2013. Discovery and characterization of two Nimrod superfamily members in *Anopheles gambiae*. Pathog. Glob. Health 107, 463–474. https://doi.org/10.1179/204777213X13867543472674.
Möller, J., Lühmann, T., Chabria, M., Hall, H., Vogel, V., 2013. Macrophages lift off surface-bound bacteria using a filopodium- lamellipodium hook-and-shovel mechanism. Sci. Rep. 3, 1–8. https://doi.org/10.1038/srep02884.
Myers, A.L., Harris, C.M., Choe, K.M., Brennan, C.A., 2018. Inflammatory production of reactive oxygen species by Drosophila hemocytes activates cellular immune defenses. Biochem. Biophys. Res. Commun. 505, 726–732. https://doi.org/10.1016/j.bbrc.2018.09.126.
Nagaosa, K., Okada, R., Nonaka, S., Takeuchi, K., Fujita, Y., Miyasaka, T., Manaka, J., Ando, I., Nakanishi, Y., 2011. Integrin βν-mediated phagocytosis of apoptotic cells in Drosophila embryos. J. Biol. Chem. 286, 25770–25777. https://doi.org/10.1074/jbc.M110.204503.
Nehme, N.T., Liégeois, S., Kele, B., Giammarinaro, P., Pradel, E., Hoffmann, J.A., Ewbank, J.J., Ferrandon, D., 2007. A model of bacterial intestinal infections in Drosophila melanogaster. PLoS Pathog. 3, 1694–1709. https://doi.org/10.1371/journal.ppat.0030173.
Nehme, N.T., Quintin, J., Cho, J.H., Lee, J., Lafarge, M.C., Kocks, C., Ferrandon, D., 2011. Relative roles of the cellular and humoral responses in the Drosophila host defense against three gram-positive bacterial infections. PLoS One 6. https://doi.org/10.1371/journal.pone.0014743.
Nickerson, D.P., Brett, C.L., Merz, A.J., 2009. Vps-C complexes: gatekeepers of endolysosomal traffic. Curr. Opin. Cell Biol. 21, 543–551. https://doi.org/10.1016/j.ceb.2009.05.007.
Nieto, C., Almendinger, J., Gysi, S., Gómez-orte, E., Kaech, A., Hengartner, M.O., Schnabel, R., Moreno, S., Cabello, J., 2010. ccz-1 Mediates the Digestion of Apoptotic Corpses in C. Elegans. https://doi.org/10.1242/jcs.062331.
Nonaka, S., Nagaosa, K., Mori, T., Shiratsuchi, A., Nakanishi, Y., 2013. Integrin ??pS3/??-mediated phagocytosis of apoptotic cells and bacteria in Drosophila. J. Biol. Chem. 288, 10374–10380. https://doi.org/10.1074/jbc.M113.451427.
O'Leary, D.D.M., Koester, S.E., 1993. Development of projection neuron types, axon pathways, and patterned connections of the mammalian cortex. Neuron 10, 991–1006. https://doi.org/10.1016/0896-6273(93)90049-W.
Paredes, J.C., Welchamn, D.P., Poidevin, M., Lemaitre, B., 2011. Negative regulation by amidase PGRPs shapes the Drosophila antibacterial response and protects the fly from innocuous infection. Immunity 35 (5), 770–779. https://doi.org/10.1016/j.immuni.2011.09.018.
Pearson, A., Lux, A., Krieger, M., 1995. Expression cloning of dSR-CI, a class C macrophage-specific scavenger receptor from Drosophila melanogaster. Proc. Natl. Acad. Sci. U. S. A 92, 4056–4060. https://doi.org/10.1073/pnas.92.9.4056.
Pearson, A.M., Baksa, K., Rämet, M., Protas, M., McKee, M., Brown, D., Ezekowitz, R.A.B., 2003. Identification of cytoskeletal regulatory proteins required for efficient phagocytosis in Drosophila. Microb. Infect. 5, 815–824. https://doi.org/10.1016/S1286-4579(03)00157-6.
Peltan, A., Briggs, L., Matthews, G., Sweeney, S.T., Smith, D.F., 2012. Identification of Drosophila gene products required for phagocytosis of leishmania donovani. PLoS One 7. https://doi.org/10.1371/journal.pone.0051831.
Peterson, C., Carney, G.E., Taylor, B.J., White, K., 2002. Reaper is required for neuroblast apoptosis during Drosophila development. Development 129, 1467.
Philips, J.A., Rubin, E.J., Perrimon, N., 2005. Drosophila RNAi Screen Reveals CD36 Family Member Required for Mycobacterial Infection, vol 309. pp. 2003–2006.
Poteryaev, D., Datta, S., Ackema, K., Zerial, M., Spang, A., 2010. Identification of the switch in early-to-late endosome transition. Cell 141, 497–508. https://doi.org/10.1016/j.cell.2010.03.011.
Poteryaev, D., Fares, H., Bruce, B., Spang, A., 2007. Caenorhabditis elegans SAND-1 is essential for RAB-7 function in endosomal traffic. EMBO J. 26, 301–312. https://doi.org/10.1038/sj.emboj.7601498.
Rämet, M., Manfruelli, P., Pearson, A., Mathey-Prevot, B., Ezekowitz, R.A.B., 2002. Functional genomic analysis of phagocytosis and identification of a Drosophila receptor for E. Coli. Nature 416, 644–648. https://doi.org/10.1038/nature735.
Rämet, M., Pearson, A., Manfruelli, P., Li, X., Koziel, H., Göbel, V., Chung, E., Krieger, M., Ezekowitz, R.A.B., 2001. Drosophila scavenger receptor CI is a pattern recognition receptor for bacteria. Immunity 15, 1027–1038. https://doi.org/10.1016/S1074-7613(01)00249-7.
Rogulja-Ortmann, A., Luer, K., Seibert, J., Rickert, C., Technau, G.M., 2007. Programmed cell death in the embryonic central nervous system of Drosophila melanogaster. Development 134, 105–116. https://doi.org/10.1242/dev.02707.
Royet, J., Gupta, D., Dziarski, R., 2011. Peptidoglycan recognition proteins: modulators of the microbiome and inflammation. Nat. Rev. Immunol. 11, 837–851. https://doi.org/10.1038/nri3089.
Samakovlis, C., Kimbrell, D. a, Kylsten, P., Engström, A., Hultmark, D., 1990. The immune response in Drosophila: pattern of cecropin expression and biological activity. EMBO

J. 9, 2969–2976.
Savill, J., Hogg, N., Ren, Y., Haslett, C., 1992. Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis. J. Clin. Investig. 90, 1513–1522. https://doi.org/10.1172/JCI116019.
Schmucker, D., Clemens, J.C., Shu, H., Worby, C.A., Xiao, J., Muda, M., Dixon, J.E., Zipursky, S.L., 2000. Drosophila Dscam is an axon guidance receptor exhibiting extraordinary molecular diversity. Cell 101, 671–684. https://doi.org/10.1016/S0092-8674(00)80878-8.
Segawa, K., Nagata, S., 2015. An apoptotic “eat me” signal: phosphatidylserine exposure. Trends Cell Biol. 25, 639–650. https://doi.org/10.1016/j.tcb.2015.08.003.
Seong, C.S., Varela-Ramirez, A., Aguilera, R.J., 2006. DNase II deficiency impairs innate immune function in Drosophila. Cell. Immunol. 240, 5–13. https://doi.org/10.1016/j.cellimm.2006.05.007.
Serizier, S.B., McCall, K., 2017. Scrambled eggs: apoptotic cell clearance by non-professional phagocytes in the Drosophila ovary. Front. Immunol. 8. https://doi.org/10.3389/fimmu.2017.01642.
Shandala, T., Lim, C., Sorvina, A., Brooks, D., 2013. A Drosophila model to image phagosome maturation. Cells 2, 188–201. https://doi.org/10.3390/cells2020188.
Shinzawa, N., Nelson, B., Aonuma, H., Okado, K., Fukumoto, S., Miura, M., Kanuka, H., 2009. p38 MAPK-dependent phagocytic encapsulation confers infection tolerance in Drosophila. Cell Host Microbe 6, 244–252. https://doi.org/10.1016/j.chom.2009.07.010.
Shiratsuchi, A., Mori, T., Sakurai, K., Nagaosa, K., Sekimizu, K., Lee, B.L., Nakanishi, Y., 2012. Independent recognition of Staphylococcus aureus by two receptors for phagocytosis in Drosophila. J. Biol. Chem. 287, 21663–21672. https://doi.org/10.1074/jbc.M111.333807.
Shklover, J., Levy-Adam, F., Kurant, E., 2015a. Apoptotic Cell Clearance in Development, first ed. Elsevier Inc Current Topics in Developmental Biology. https://doi.org/10.1016/bs.ctdb.2015.07.024.
Shklover, J., Levy-Adam, F., Kurant, E., 2015b. Apoptotic Cell Clearance in Development, first ed. Elsevier Inc Current Topics in Developmental Biology. https://doi.org/10.1016/bs.ctdb.2015.07.024.
Shklyar, B., Levy-Adam, F., Mishnaevski, K., Kurant, E., 2013. Caspase activity is required for engulfment of apoptotic cells. Mol. Cell Biol. 33, 3191–3201. https://doi.org/10.1128/MCB.00233-13.
Shklyar, B., Sellman, Y., Shklover, J., Mishnaevski, K., Levy-Adam, F., Kurant, E., 2014. Developmental regulation of glial cell phagocytic function during Drosophila embryogenesis. Dev. Biol. 393, 255–269. https://doi.org/10.1016/j.ydbio.2014.07.005.
Stenbak, C.R., Ryu, J.-H., Leulier, F., Pili-Floury, S., Parquet, C., Herve, M., Chaput, C., Boneca, I.G., Lee, W.-J., Lemaitre, B., Mengin-Lecreulx, D., 2004. Peptidoglycan molecular requirements allowing detection by the Drosophila immune deficiency pathway. J. Immunol. 173, 7339–7348. https://doi.org/10.4049/jimmunol.173.12.7339.
Stroschein-Stevenson, S.L., Foley, E., O'Farrell, P.H., Johnson, A.D., 2006. Identification of Drosophila gene products required for phagocytosis of Candida albicans. PLoS Biol. 4, 0087–0099. https://doi.org/10.1371/journal.pbio.0040004.
Stuart, L.M., Boulais, J., Charriere, G.M., Hennessy, E.J., Brunet, S., Jutras, I., Goyette, G., Rondeau, C., Letarte, S., Huang, H., Ye, P., Morales, F., Kocks, C., Bader, J.S., Desjardins, M., Ezekowitz, R.A.B., 2007. A systems biology analysis of the Drosophila phagosome. Nature 445, 95–101. https://doi.org/10.1038/nature05380.
Stuart, L.M., Deng, J., Silver, J.M., Takahashi, K., Tseng, A.A., Hennessy, E.J., Ezekowitz, R.A.B., Moore, K.J., 2005. Response to Staphylococcus aureus requires CD36-mediated phagocytosis triggered by the COOH-terminal cytoplasmic domain. J. Cell Biol. 170, 477–485. https://doi.org/10.1083/jcb.200501113.
Tasdemir-Yilmaz, O.E., Freeman, M.R., 2014. Astrocytes engage unique molecular programs to engulf pruned neuronal debris from distinct subsets of neurons. Genes Dev. 28, 20–33. https://doi.org/10.1101/gad.229518.113.
Theopold, U., Krautz, R., Dushay, M.S., 2014. The Drosophila clotting system and its messages for mammals. Dev. Comp. Immunol. 42, 42–46. https://doi.org/10.1016/j.dci.2013.03.014.
Timmons, A.K., Mondragon, A.A., Schenkel, C.E., Yalonetskaya, A., Taylor, J.D., Moynihan, K.E., Etchegaray, J.I., Meehan, T.L., McCall, K., 2016. Phagocytosis genes nonautonomously promote developmental cell death in the *Drosophila* ovary. Proc. Natl. Acad. Sci. Unit. States Am. 113, E1246–E1255. https://doi.org/10.1073/pnas.1522830113.
Tissot, M., Stocker, R.F., 2000. Metamorphosis in Drosophila and other insects: the fate of neurons throughout the stages. Prog. Neurobiol. 62, 89–111. https://doi.org/10.1016/s0301-0082(99)00069-6.
Togane, Y., Ayukawa, R., Hara, Y., Akagawa, H., Iwabuchi, K., Tsujimura, H., 2012. Spatio-temporal pattern of programmed cell death in the developing Drosophila optic lobe. Dev. Growth Differ. 54, 503–518. https://doi.org/10.1111/j.1440-169X.2012.01340.x.
Tung, T.T., Nagaosa, K., Fujita, Y., Kita, A., Mori, H., Okada, R., Nonaka, S., Nakanishi, Y., 2013. Phosphatidylserine recognition and induction of apoptotic cell clearance by Drosophila engulfment receptor Draper. J. Biochem. 153, 483–491. https://doi.org/10.1093/jb/mvt014.
Vanha-aho, L.M., Valanne, S., Rämet, M., 2016. Cytokines in Drosophila immunity. Immunol. Lett. 170, 42–51. https://doi.org/10.1016/j.imlet.2015.12.005.
Vieira, O.V., Botelho, R.J., Rameh, L., Brachmann, S.M., Matsuo, T., Davidson, H.W., Schreiber, A., Backer, J.M., Cantley, L.C., Grinstein, S., 2001. Distinct Roles of Class I and Class III Phosphatidylinositol 3-kinases in Phagosome Formation and Maturation 19–25. https://doi.org/10.1083/jcb.200107069.
Vieira, O.V., Bucci, C., Harrison, R.E., Trimble, W.S., Lanzetti, L., Gruenberg, J., Schreiber, A.D., Stahl, P.D., Grinstein, S., 2003. Modulation of Rab5 and Rab7 Recruitment to Phagosomes by Phosphatidylinositol 3-Kinase, vol 23. pp. 2501–2514. https://doi.org/10.1128/MCB.23.7.2501.
Wang, C.W., Stromhaug, P.E., Shima, J., Klionsky, D.J., 2002. The Ccz1-Mon1 protein complex is required for the late step of multiple vacuole delivery pathways. J. Biol. Chem. 277, 47917–47927. https://doi.org/10.1074/jbc.M208191200.
Watson, F.L., Pu, R., Thomas, F., Lamar, D.L., Hughes, M., Kondo, M., Rebel, V.I., Schmucker, D., 2005. Extensive diversity of Ig-superfamily proteins in the immune system of insects. Science 309, 1874–1879.
Weavers, H., Evans, I.R., Martin, P., Wood, W., 2016. Corpse engulfment generates a molecular memory that primes the macrophage inflammatory response. Cell 165, 1658–1671. https://doi.org/10.1016/j.cell.2016.04.049.
Wojtowicz, W.M., Flanagan, J.J., Millard, S.S., Zipursky, S.L., Clemens, J.C., 2004. Alternative splicing of Drosophila Dscam generates axon guidance teceptors that exhibit isoform-specific homophilic binding. Cell 118, 619–633.
Woodcock, K.J., Kierdorf, K., Pouchelon, C.A., Vivancos, V., Dionne, M.S., Geissmann, F., 2015. Macrophage-derived upd3 cytokine causes impaired glucose homeostasis and reduced lifespan in Drosophila fed a lipid-rich diet. Immunity 42, 133–144. https://doi.org/10.1016/j.immuni.2014.12.023.
Yeung, T., Ozdamar, B., Paroutis, P., Grinstein, S., 2006. Lipid metabolism and dynamics during phagocytosis. Curr. Opin. Cell Biol. 18, 429–437. https://doi.org/10.1016/j.ceb.2006.06.006.
Yousefian, J., Troost, T., Grawe, F., Sasamura, T., Fortini, M., Klein, T., 2013. Dmon1 controls recruitment of Rab7 to maturing endosomes in Drosophila. J. Cell Sci. 126, 1583–1594. https://doi.org/10.1242/jcs.114934.
Zhan, X.L., Clemens, J.C., Neves, G., Hattori, D., Flanagan, J.J., Hummel, T., Vasconcelos, M.L., Chess, A., Zipursky, S.L., 2004. Analysis of Dscam diversity in regulating axon guidance in Drosophila mushroom bodies. Neuron 43, 673–686. https://doi.org/10.1016/j.neuron.2004.07.020.
Ziegenfuss, J.S., Biswas, R., Avery, M.A., Hong, K., Sheehan, A.E., Yeung, Y.G., Stanley, E.R., Freeman, M.R., 2008. Draper-dependent glial phagocytic activity is mediated by Src and Syk family kinase signalling. Nature 453, 935–939. https://doi.org/10.1038/nature06901.
Zsámboki, J., Csordás, G., Honti, V., Pintér, L., Bajusz, I., Galgóczy, L., Andó, I., Kurucz, É., 2013. Drosophila Nimrod proteins bind bacteria. Cent. Eur. J. Biol. 8, 633–645. https://doi.org/10.2478/s11535-013-0183-4.