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Signaling and membrane dynamics during phagocytosis: many roads lead to the phagos(R)ome

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Phagocytosis is the mechanism used by specialized cells such as macrophages, dendritic cells and neutrophils to internalize, degrade and eventually present peptides derived from particulate antigens. This process relies on profound rearrangements of the actin cytoskeleton and the plasma membrane to engulf particles. Recent work has highlighted the early recruitment of internal membranes derived from endocytic compartments and from the endoplasmic reticulum to allow plasma membrane extension at the onset of phagocytosis. This ensures that the phagosome is rapidly provided with the machinery appropriate for later phagocytic functions, including particle degradation and antigen presentation.

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Abbreviations

ARF	ADP ribosylation factor
CR	complement receptor
ER	endoplasmic reticulum
FcR	Fc receptor
GEF	guanine nucleotide exchange factor
Ig	immunoglobulin
ITAM	immunoreceptor tyrosine-based activation motif
MHC	major histocompatibility complex
PH domain	pleckstrin homology domain
PI3K	phosphatidylinositol 3′-kinase
PI3P	phosphatidylinositol 3-phosphate
PIP2	phosphatidylinositol 4,5-bisphosphate
PIP3	phosphatidylinositol 3,4,5-trisphosphate
PIPKIα	phosphatidylinositol-4-phosphate 5-kinase Iα
RNAi	RNA interference
SNARE	soluble N-ethylmaleimide-sensitive factor attachment protein receptors
TAP	transporter associated with antigen processing
VAMP	vesicle-associated membrane protein
WASP	Wiscott–Aldrich syndrome protein

Introduction

In mammals, phagocytosis is a hallmark of macrophages, dendritic cells and polymorphonuclear neutrophils, all of which play a crucial role in inflammatory and immune responses [1,2]. In addition to these professional phagocytes, many cell types are also able to phagocytose targets, for example when dead neighboring cells need to be cleared off [3]. Lower eukaryotes such as the mold *Dictyostelium discoideum* use phagocytosis for feeding. Phagocytosis is triggered by engagement of receptors on the surface of the phagocyte after recognition of cognate ligands on the particle. Among a variety of phagocytic receptors, including receptors for mannose residues, phosphatidylserine and C-reactive protein, the best-characterized are receptors for opsonins such as immunoglobulins (Ig) and complement. Signal transduction leads to actin polymerization, which is thought to be the driving force for membrane extension around the particle. To accommodate pseudopod extension, membranes from internal stores are recruited to sites of phagocytosis at the plasma membrane. Phagocytosis ultimately leads to degradation of internalized particles as a result of the activity of lytic enzymes and the phagocyte oxidase complex.

In this review, we will discuss recent data describing the signaling network downstream of receptor triggering, the biology of phagosome formation and maturation, and recent developments concerning antigen presentation, with a focus on macrophages and dendritic cells.

Signaling and actin: the family picture

Signals downstream of FcRs

Ligation and clustering of the Fcγ receptors triggers actin polymerization, which remains restricted to the site of particle attachment and is biphasic: the actin cup forms within seconds upon receptor engagement and disassembles within one to five minutes (first reported by [4] and recently revisited by [5–7]). Src-family kinases are activated upon receptor ligation and phosphorylate the immunoreceptor tyrosine-based activation motif (ITAM) of the cytoplasmic regions of the clustered FcγRs. Phosphorylated ITAMs serve as binding sites for a variety of cytosolic enzymes and adaptors including the tyrosine kinase Syk and type I phosphatidylinositol 3′-kinases (PI3Ks), which are required for phagocytosis. These, in turn, trigger the tyrosine phosphorylation of multiple proteins and the local accumulation of PIP3, respectively (for review see [8–10]). Control of actin filament polymerization in the phagocytic cup depends on Cdc42 and Rac, two Rho family GTPases that have recently been shown to be rapidly and transiently activated during FcR phagocytosis [11,12•] (for review see [13]). In addition, the ADP-ribosylation factor (ARF)6 GTP-binding

protein is activated by GTP loading during FcR phagocytosis, displaying similar kinetics, which raises the possibility that members of the Rho and ARF subgroups act cooperatively during phagocytosis [11]. However, there are still gaps in our understanding of the cascade of events leading to Rho and ARF family GTPase activation during phagocytosis. Vav, a Dbl-family Rho GEF (guanine nucleotide exchange factor), was recently identified as a Rac activator during FcR phagocytosis ([12•]), but the GEFs responsible for Cdc42 and ARF6 activation remain unknown. The nucleotide exchange activity of Rho and ARF6 GEFs can be modulated by tyrosine phosphorylation and/or binding of their pleckstrin homology (PH) domains to phosphoinositides (for review see [14,15]). However, the rise in PIP3 production that occurs at the onset of phagocytosis does not appear to play any role in activation, as GTP loading on Rac and ARF6 is not affected by the PI3K inhibitor wortmannin (Niedergang and Chavrier, unpublished). PIP3-independent activation of Rac is in line with the previous observation that PI3K activity is not required for actin filament assembly underneath the phagocytic cup [16]. ARF6 activity, although required for engulfment, does not control actin assembly and is instead involved in focal delivery of recycling membranes at the site of phagocytosis ([11,17] and see below).

In their GTP-bound conformation, Rac and Cdc42 interact with downstream effectors that are directly involved in the regulation of actin filament assembly, such as the Wiscott–Aldrich syndrome protein (WASP), which is recruited to the nascent phagocytic cup by interacting with GTP-bound Cdc42 [18,19]. In turn, WASP interacts with and activates the actin-nucleating Arp2/3 complex, which is necessary for the assembly of actin filaments at phagocytic cups [20]. In addition, a complex consisting of WASP, the adaptor proteins Fyb/SLAP, Nck and SLP-76, and the cytoskeletal Ena/VASP proteins is assembled during FcγR phagocytosis and localizes to the forming cup [19]. Recruitment of VASP to the phagocytic cup requires the activity of Rho proteins [19], and may also involve a direct interaction with WASP [18]. Although the precise role of Ena/VASP proteins is still a matter of debate, these proteins are clearly involved in actin dynamics and recruitment of VASP to the nascent cup is essential for engulfment [19,21].

Phosphoinositide signaling in the phagocytic cup

Phosphoinositide kinases and phosphatases, which are known effectors of Rho GTPases, may also participate in the regulation of phagocytosis downstream of Rho. Biphasic accumulations of PIP2 and PIP3 that are confined to the phagocytic cup have been documented using specific lipid-binding domains fused with GFP (for review see [8]). Interestingly, PIPK-Iα and the p110β-catalytic subunit of PI3K, which synthesize PIP2 and PIP3 respectively, are enriched in the cup and inhibition of their activity leads to a significant reduction of FcR phagocytosis in macrophages [6,22]. Loss of PIP2, which coincides with phagosome closure, involves PLCγ, whereas control of PIP3 levels is achieved by SHIP, an SH2-domain-containing inositol 5′-phosphatase that also localizes to the cup [23,24]. The observation that inhibition of PIPK-Iα interferes with actin filament assembly in the cup is a strong indication that PIP2 is involved in actin remodeling during phagocytosis, probably through its regulation of many cytoskeletal proteins such as WASP ([6] and references therein). These data contrast with reports of persistent actin polymerization in the absence of PIP3 accumulation at the cup (i.e. in the presence of PI3K inhibitors), leading to the conclusion that PI3K products are instead involved in membrane pseudopod extension (recently reviewed in [8,10]). Myosin-X, which is recruited to the phagocytic cup through binding of PI3K products by its PH domain, is a key downstream effector of PI3K required for optimal extension of pseudopods during FcR phagocytosis [25•]. Other myosin motors are also involved in the generation of contractility in the phagocytic cup, which is necessary for particle engulfment and phagosome closure [26•,27,28]. Similarly, the adaptor protein Gab2, which is targeted to the cup through its PH domain, may further contribute to PIP3 production by interacting with PI3K [29].

Signaling via the complement receptors

Complement receptor (CR)3-mediated phagocytosis, initiated by recognition of complement fragment C3bi by αM/β2 integrins (also known as CR3 or Mac1) on the phagocytic cell surface, utilizes RhoA, and not Cdc42/Rac [30]. Recently, we have become aware of additional players in the pathway with the finding that the RhoA effector Rho-kinase (ROK) and its downstream target myosin-II regulate the Arp2/3 complex and F-actin accumulation [26•]. In addition, activation of CR3 requires extra stimulation called inside-out signaling, which results in conformational change of the integrin receptors. Integrin activation is taken over by the small GTP-binding protein Rap1 (for review see [31]). A recent report demonstrated that FcR ligation may be one such signal; it impinges on CR3's capability to bind C3bi-coated targets by promoting clustering of CR3 into high-avidity complexes [32]. What these findings also illustrate is that phagocytosis mediated by either FcR or CR alone is very unlikely to occur, and that phagocytosis will rather be the consequence of multiple engagements of various types of receptors that cooperate to ensure efficient clearing of pathogens and cell debris.

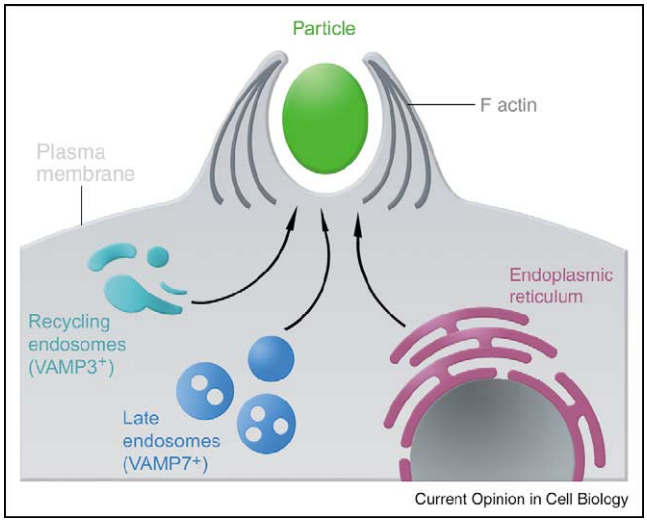
Focal exocytosis during phagocytosis

Until recently, it was assumed that phagosomes formed simply from sealing of the plasma membrane around the particle and that unfolding of plasma membrane ruffles would be sufficient to extend the membrane around the particle. Capacitance measurements however indicated

that the surface of the plasma membrane, instead of decreasing as a result of massive internalization of particles, is instead increased [33]. Grinstein and colleagues showed that the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) fusion machinery was necessary for phagocytosis to proceed, supporting the notion that internal membranes are recruited to and fuse with the plasma membrane at the site of phagocytosis in a process called 'focal exocytosis' [34]. Several intracellular compartments contribute to the formation of the phagosome, including recycling endosomes [35], the endoplasmic reticulum (ER) [36•], and late endosomes (V Braun and F Niedergang, unpublished data). Recycling endosomes bearing transferrin receptors and the v-SNARE VAMP3 (vesicle-associated membrane protein 3) fuse with phagosomes before closure [35]. Direct fusion of the ER and forming phagosomes has been observed by Desjardins and colleagues [36•]. Last but not least, we have further shown that VAMP7/TI-VAMP-positive late endocytic compartments are exocytosed at the site of phagosome formation (V Braun and F Niedergang, unpublished; see Figure 1). Why and how so many intracellular compartments contribute to the formation of a 'mosaic' phagosome has now to be investigated. In addition to providing membranes, these internal compartments also deliver specific proteins that build the phagosome and initiate its maturation.

Focal exocytosis of intracellular membranes relies on several steps, including vesicle budding from a donor compartment, transport along cytoskeletal tracks and fusion of vesicle with a receptor compartment. A role for the clathrin adaptor AP1 has recently been highlighted during phagocytosis in *D. discoideum* [37]. This is intriguing in the light of the recent results showing that clathrin is not involved in phagocytosis in macrophages [38] or in *Drosophila* S2 cells [39•], although a previous study showed that phagocytosis was inhibited in alveolar macrophages by antibodies directed against clathrin [40]. Deletion of the εCOP subunit of the COPI coat in macrophages results in deficient phagocytosis and impaired VAMP3-positive endomembrane recruitment [41]. Consistent with this, depletion of β- or δCOP by RNAi leads to a defect in phagocytosis in *Drosophila* [39•]. COPI recruitment and coatomer assembly depends on the small GTP-binding protein ARF1, and is sensitive to BFA, an inhibitor of the GTP exchange factors of the Arf family. Although it was initially reported that BFA had no effect on phagocytosis [17], more prolonged treatment of the cells with this drug depressed phagocytosis [41]. All these results are indicative of an indirect role for ARF/COPI in homeostasis of the intracellular compartments rather than a direct role in vesicle budding from recycling endosomes to sites of phagocytosis. Dynamin II is recruited to nascent phagosomes via its interaction with amphiphysin IIm, and both proteins (but not dynamin I) are required for phagosome formation [38,42]. Dynamin II, which is located on tubular recycling endosomes [43], was shown to participate in vesicle budding and fission from internal compartments during focal exocytosis in phagocytosing macrophages [44•]. Additional roles for dynamin II in actin remodeling in the phagocytic cup can also be envisaged.

Figure 1

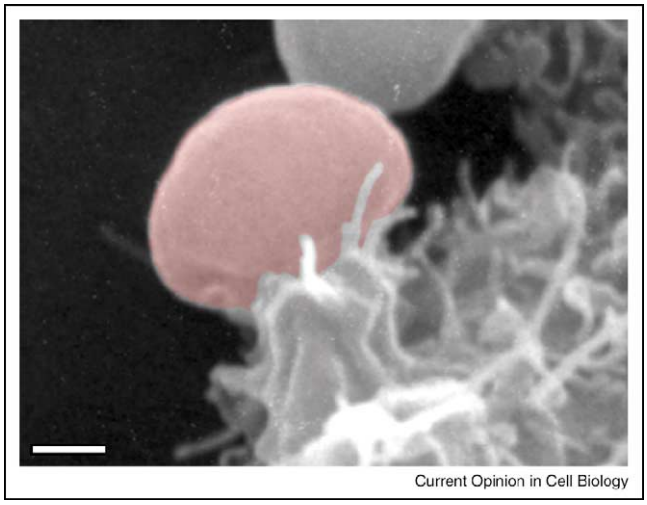


Focal exocytosis during phagocytosis. Many intracellular compartments are recruited to the plasma membrane at the onset of phagocytosis: recycling endosomes bearing the VAMP3 protein [35], late endosomal compartment expressing TI-VAMP/VAMP7 (Braun and Niedergang, unpublished) and the ER [36•], to which no fusion complex has been functionally linked so far. The relative contribution of all these compartments to phagosome formation is still unclear.

Vesicle transport to the plasma membrane is poorly characterized. Microtubules and actin microfilaments are necessary for the early steps of phagosome formation [45]. Several myosin motors, including myosin X, that are recruited to the phagosome could play a role in phagosome formation ([28], and see above).

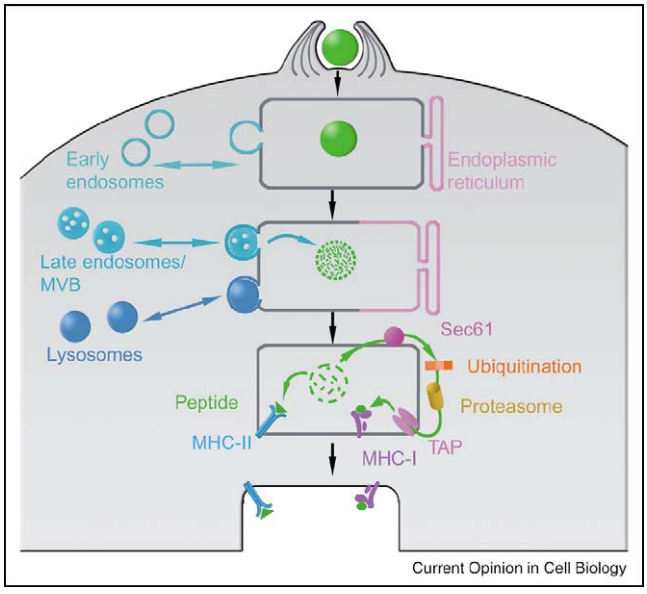
Fusion of internal membranes to the site of phagocytosis may require SNARE-dependent machineries under the control of Rab GTPases. A role for VAMP3 in the delivery of recycling endosomes has been proposed [34]. However, phagocytosis of opsonized particles was normal in macrophages from mice deficient in VAMP3, suggesting that compensatory mechanisms may exist [46]. In addition, we have shown that the SNARE protein VAMP7/TI-VAMP controls exocytosis of the late endocytic compartment and is required for phagosome formation (V Braun and F Niedergang, unpublished data). Notably, VAMP7 is part of a complex containing syntaxin 7, syntaxin 8 and Vti1 that is involved in macropinocytosis in *D. discoideum*, a process related to phagocytosis [47]. So far, it has not been possible to demonstrate that ER fusion is necessary for phagocytosis and that this process relies

Figure 2



ARF6 activity is required for membrane extension during phagocytosis. Scanning electron micrograph of RAW264.7 macrophages expressing a dominant-negative mutant of ARF6 (ARF6-T27N) and incubated with IgG-opsonized red blood cells for 60 min at 37°C. In these cells, phagocytosis is blocked as a result of inhibition of focal recycling of VAMP3-positive membranes and impaired membrane extension around particles, although actin polymerization still occurs [11]. It is interesting to note that phagocytosis is blocked at a similar stage when PI3K, dynamin II, amphiphysin Iim or myosin X are inactivated, suggesting that all these proteins contribute to pseudopod extension. Scale bar, 1 μm.

Figure 3



Phagosome maturation and antigen processing and presentation. The phagosome undergoes a series of fusion events with the endocytic compartments (early endosomes, late endosomes, multivesicular bodies [MVBs] and lysosomes). Peptides generated from the degradation of the internalized particle are then loaded on MHC class II (MHC-II) molecules, mainly acquired from endocytic compartments storing newly synthesized MHC-II (MVB). Fusion with the ER delivers the MHC class I (MHC-I) presentation machinery to the phagosome [65••–67••]. Peptides generated by degradation in acidic phagolysosome are exported to the cytosol via the Sec61 complex, ubiquitinated, processed by the proteasome and transported back via TAP into the phagosome lumen where they are eventually loaded onto MHC-I molecules. Peptide:MHC-I/II complexes are then exported to the cell surface by a pathway that is poorly defined at the molecular level.

on SNARE recognition. An interaction of ER Sec22 with plasma membrane Sso1/Sec9c, recently documented in yeast, may be the subject of further investigations in the context of phagocytosis [48].

The role of the small GTP-binding proteins of the Rab and Arf families in phagosome formation has been investigated. The small GTP-binding protein ARF6, and presumably also Rab11, control recruitment of membranes from recycling endosomes to nascent phagosomes [11,49] (Figure 2). Interestingly, recently characterized effectors common to Rab11 and ARF6 called arfophilins could be a link between ARF6 and Rab11 pathways [50]. Rab5 (which controls homotypic early endosome fusion) and Rab7 (which regulates trafficking to and from late endosomes) do not participate in phagosome formation, but rather play a role in its maturation (see below and [38,51,52].

Phagosome maturation

After closure, the phagosome matures via a series of fusion events with organelles of the endocytic pathway (Figure 3). Ultimately, this leads to the insertion of lysosomal membrane proteins (LIMPII, Lamps) in the phagosomal membrane and to the delivery of lysosomal lytic enzymes into the lumen of the phagosome [7,53,54]. In the endocytic pathway, fusion and fission events are regulated by Rab and SNAREs proteins and it is assumed that phagosome maturation is similarly orchestrated, although the role of these proteins in phagosome maturation has only recently started to be studied. Syntaxin 13, located on recycling endosomes, and syntaxin 7, present on late endosomes, are involved in phagosome maturation [51]. Rab7 is required for phagosome maturation in *D. discoideum* [55] and in macrophages [56]. Interestingly, in the latter case Rab5 is necessary for Rab7 recruitment and function [52]. Accumulation of PI3P produced by VPS34 type III PI3K on the phagosomal membrane occurs soon after phagosome closure and is essential for phagosome maturation [57,58]. The role of PI3P could be mediated by FYVE-domain-containing proteins such as Hrs1, whose depletion arrests phagosomal maturation at an early stage [58] (Viera *et al.*, unpublished). PI3P synthesis on the phagosomal membrane may be regulated by Rab5, which interacts with VPS34 PI3K [59].

During the course of maturation, phagosomes migrate on microtubules from the cell periphery to a perinuclear

location. A Rab7 effector, the Rab7-interacting lysosomal protein (RILP), which interacts with the dynein–dynactin complex, may be the link between the migrating phagosomes and microtubules [56]. Actin nucleation, observed on phagosomes, might allow migration, contact and eventually fusion of phagosomes with late endocytic compartments [60,61].

The phagosomal content may ultimately be recycled to the outside of phagocytes and this property, long considered a peculiarity of *D. discoideum*, has recently been observed in macrophages, where egestion of polystyrene beads is under the control of syntaxin 3 [62]. Microtubules participate in the egestion process, whereas cortical actin prevents it [45].

Antigen presentation

Hydrolases delivered to the phagosomal lumen by late endosomes and lysosomes contribute to the degradation of the ingested material. Peptides derived from processing are then loaded onto major histocompatibility complex (MHC) class II molecules, originating mostly from newly synthetized pools stored in endocytic compartments (late endosomes or multivesicular bodies, as well as lysosomes or lamellar bodies). MHC class II molecules are then expressed on the surface of the phagocytic cell to activate $CD4^+$ T lymphocytes (for a review, see [63]). Phagocytosed antigens can also be presented by MHC class I molecules and activate cytotoxic $CD8^+$ T lymphocytes in a process called 'cross-presentation' (for a review see [64]). Peptide loading on MHC class I molecules normally occurs in the ER after translocation of the peptides processed via the cytosolic degradative proteasome complex (for a review see [64]). Recently, a missing link between exogenous antigens and the MHC class I presentation machinery has been unraveled. Indeed, fusion of phagosomes with the ER provides this organelle with components of the class I presentation machinery: Sec61 transporters, proteasome subunits and TAP (transporter associated with antigen presentation) proteins have been found associated with the phagosome [65••-67••]. These data suggest that an antigen is retrotranslocated through the Sec61 channel to the cytosol, where it undergoes proteasomal degradation and then remains in the vicinity of the phagosome it originates from; it is next translocated back to the phagosomal lumen via TAP transporters, and is eventually loaded on MHC class I molecules (Figure 3). This set of data sheds new light on the mechanism of cross-presentation of phagocytosed material [64].

Conclusions

This review focuses on recent data concerning phagocytosis in macrophages and dendritic cells. The mechanisms of phagocytosis might differ slightly in other professional phagocytes, such as polymorphonuclear neutrophils (for a review, see [68]) and in non-professional cells ingesting apoptotic debris (for a review, see [3]). New insights can be expected in the coming years from global analyses of phagosomes by proteomics in macrophages and in *D. discoideum*, as well as from RNAi-based screening in *Drosophila* [39•,53,69]. It will then be possible to analyze how fine-tuning of phagocytosis is achieved when multiple receptors are simultaneously engaged under physiological situations. Unraveling the mechanisms of phagosome formation and maturation would provide a unique opportunity to understand the coordination between actin and membrane dynamics, which are also crucial for other cell functions including cell migration and cell–cell adhesion.

Update

New light has been shed recently on the cooperation between receptors on the surface of macrophages. This work showed that engagement of Toll-like receptors (TLRs), known to induce pro-inflammatory signals, also influences the maturation of phagosomes via a cascade involving the MyD88 adaptor and p38 mitogen-activated protein kinase [70••].

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Aderem A: **Phagocytosis and the inflammatory response**. *J Infect Dis* 2003, **187**:S340-S345.
2. Greenberg S, Grinstein S: **Phagocytosis and innate immunity**. *Curr Opin Immunol* 2002, **14**:136-145.
3. Grimsley C, Ravichandran KS: **Cues for apoptotic cell engulfment: eat-me, don't-eat-me and come-get-me signals**. *Trends Cell Biol* 2003, **13**:648-656.
4. Greenberg S, el Khoury J, di Virgilio F, Kaplan EM, Silverstein SC: **Ca^{2+}-independent F-actin assembly and disassembly during Fc receptor-mediated phagocytosis in mouse macrophages**. *J Cell Biol* 1991, **113**:757-767.
5. Diakonova M, Bokoch G, Swanson JA: **Dynamics of cytoskeletal proteins during Fcγ-receptor-mediated phagocytosis in macrophages**. *Mol Biol Cell* 2002, **13**:402-411.
6. Coppolino MG, Dierckman R, Loijens J, Collins RF, Pouladi M, Jongstra-Bilen J, Schreiber AD, Trimble WS, Anderson R, Grinstein S: **Inhibition of phosphatidylinositol-4-phosphate 5-kinase Iα impairs localized actin remodeling and suppresses phagocytosis**. *J Biol Chem* 2002, **277**:43849-43857.
7. Henry RM, Hoppe AD, Joshi N, Swanson JA: **The uniformity of phagosome maturation in macrophages**. *J Cell Biol* 2004, **164**:185-194.
8. Brumell JH, Grinstein S: **Role of lipid-mediated signal transduction in bacterial internalization**. *Cell Microbiol* 2003, **5**:287-297.
9. May RC, Machesky LM: **Phagocytosis and the actin cytoskeleton**. *J Cell Sci* 2001, **114**:1061-1077.

10. Stephens L, Ellson C, Hawkins P: **Roles of PI3Ks in leukocyte chemotaxis and phagocytosis**. *Curr Opin Cell Biol* 2002, **14**:203-213.

11. Niedergang F, Colucci-Guyon E, Dubois T, Raposo G, Chavrier P: **ADP ribosylation factor 6 is activated and controls membrane delivery during phagocytosis in macrophages**. *J Cell Biol* 2003, **161**:1143-1150.

12. • Patel JC, Hall A, Caron E: **Vav regulates activation of rac but not Cdc42 during FcγR-mediated phagocytosis**. *Mol Biol Cell* 2002, **13**:1215-1226.
This study identifies the DH-PH-domain-containing guanine nucleotide exchange factor Vav as being responsible for activation of Rac1 during FcγR-mediated phagocytosis.

13. Chimini G, Chavrier P: **Function of Rho family proteins in actin dynamics during phagocytosis and engulfment**. *Nat Cell Biol* 2000, **2**:E191-E196.

14. Bustelo XR: **Regulatory and signaling properties of the Vav family**. *Mol Cell Biol* 2000, **20**:1461-1477.

15. Donaldson JG, Jackson CL: **Regulators and effectors of the ARF GTPases**. *Curr Opin Cell Biol* 2000, **12**:475-482.

16. Cox D, Tseng CC, Bjekic G, Greenberg S: **A requirement for phosphatidylinositol 3-kinase in pseudopod extension**. *J Biol Chem* 1999, **274**:1240-1247.

17. Zhang Q, Cox D, Tseng C-C, Donaldson JG, Greenberg S: **A requirement for ARF6 in Fcγ receptor-mediated phagocytosis in macrophages**. *J Biol Chem* 1998, **273**:19977-19981.

18. Castellano F, Le Clainche C, Patin D, Carlier MF, Chavrier P: **A WASp–VASP complex regulates actin polymerization at the plasma membrane**. *EMBO J* 2001, **20**:5603-5614.

19. Coppolino MG, Krause M, Hagendorff P, Monner DA, Trimble W, Grinstein S, Wehland J, Sechi AS: **Evidence for a molecular complex consisting of Fyb/SLAP, SLP-76, Nck, VASP and WASP that links the actin cytoskeleton to Fcγ receptor signalling during phagocytosis**. *J Cell Sci* 2001, **114**:4307-4318.

20. May RC, Caron E, Hall A, Machesky LM: **Involvement of the Arp2/3 complex in phagocytosis mediated by FcγR or CR3**. *Nat Cell Biol* 2000, **2**:246-248.

21. Krause M, Dent EW, Bear JE, Loureiro JJ, Gertler FB: **Ena/VASP proteins: regulators of the actin cytoskeleton and cell migration**. *Annu Rev Cell Dev Biol* 2003, **19**:541-564.

22. Leverrier Y, Okkenhaug K, Sawyer C, Bilancio A, Vanhaesebroeck B, Ridley AJ: **Class I phosphoinositide 3-kinase p110γ is required for apoptotic cell and Fcγ receptor-mediated phagocytosis by macrophages**. *J Biol Chem* 2003, **278**:38437-38442.

23. Marshall JG, Booth JW, Stambolic V, Mak T, Balla T, Schreiber AD, Meyer T, Grinstein S: **Restricted accumulation of phosphatidylinositol 3-kinase products in a plasmalemmal subdomain during Fcγ-receptor-mediated phagocytosis**. *J Cell Biol* 2001, **153**:1369-1380.

24. Cox D, Dale BM, Kashiwada M, Helgason CD, Greenberg S: **A regulatory role for Src homology 2 domain-containing inositol 5′-phosphatase (SHIP) in phagocytosis mediated by Fcγ receptors and complement receptor 3 (αMβ2; CD11b/CD18)**. *J Exp Med* 2001, **193**:61-71.

25. • Cox D, Berg JS, Cammer M, Chinegwundoh JO, Dale BM, Cheney RE, Greenberg S: **Myosin X is a downstream effector of PI(3)K during phagocytosis**. *Nat Cell Biol* 2002, **4**:469-477.
This paper shows that myosin X, an unconventional myosin, acts as a downstream effector of PI3K through its PH domain interacting with PIP3 during FcR phagocytosis, presumably by controlling pseudopod extension.

26. • Olazabal IM, Caron E, May RC, Schilling K, Knecht DA, Machesky LM: **Rho-kinase and myosin-II control phagocytic cup formation during CR, but not FcγR, phagocytosis**. *Curr Biol* 2002, **12**:1413-1418.
This is the first demonstration that Rho-kinase and its downstream target myosin-II control the formation of the phagocytic cup during CR-mediated phagocytosis by regulating the accumulation of Arp2/3 complex and F-actin at the phagocytic site.

27. Chavrier P: **May the force be with you: myosin-X in phagocytosis**. *Nat Cell Biol* 2002, **4**:E169-E171.

28. Araki N, Hatae T, Furukawa A, Swanson JA: **Phosphoinositide-3-kinase-independent contractile activities associated with Fcγ-receptor-mediated phagocytosis and macropinocytosis in macrophages**. *J Cell Sci* 2003, **116**:247-257.

29. Gu H, Botelho RJ, Yu M, Grinstein S, Neel BG: **Critical role for scaffolding adapter Gab2 in FcγR-mediated phagocytosis**. *J Cell Biol* 2003, **161**:1151-1161.

30. Caron E, Hall A: **Identification of two distinct mechanisms of phagocytosis controlled by different Rho GTPases**. *Science* 1998, **282**:1717-1721.

31. Caron E: **Cellular functions of the Rap1 GTP-binding protein: a pattern emerges**. *J Cell Sci* 2003, **116**:435-440.

32. Jongstra-Bilen J, Harrison R, Grinstein S: **Fcγ-receptors induce Mac-1 (CD11b/CD18) mobilization and accumulation in the phagocytic cup for optimal phagocytosis**. *J Biol Chem* 2003, **278**:45720-45729.

33. Holevinsky KO, Nelson DJ: **Membrane capacitance changes associated with particle uptake during phagocytosis in macrophages**. *Biophys J* 1998, **75**:2577-2586.

34. Booth JW, Trimble WS, Grinstein S: **Membrane dynamics in phagocytosis**. *Semin Immunol* 2001, **13**:357-364.

35. Bajno L, Peng X-R, Schreiber AD, Moore H-P, Trimble WS, Grinstein S: **Focal exocytosis of VAMP3-containing vesicles at sites of phagosome formation**. *J Cell Biol* 2000, **149**:697-705.

36. • Gagnon E, Duclos S, Rondeau C, Chevet E, Cameron PH, Steele-Mortimer O, Paiement J, Bergeron JJ, Desjardins M: **Endoplasmic-reticulum-mediated phagocytosis is a mechanism of entry into macrophages**. *Cell* 2002, **110**:119-131.
This paper documents a direct fusion between the ER membrane and the forming phagosome in macrophages.

37. Lefkir Y, Malbouyres M, Gotthardt D, Ozinsky A, Cornillon S, Bruckert F, Aderem AA, Soldati T, Cosson P, Letourneur F: **Involvement of the AP-1 adaptor complex in early steps of phagocytosis and macropinocytosis**. *Mol Biol Cell* 2004, **15**:861-869.

38. Tse SML, Furuya W, Gold ES, Schreiber AD, Sandvig K, Inman RD, Grinstein S: **Differential role of actin, clathrin, and dynamin in Fcγ-receptor-mediated endocytosis and phagocytosis**. *J Biol Chem* 2003, **278**:3331-3338.

39. • Ramet M, Manfruelli P, Pearson A, Mathey-Prevot B, Ezekowitz RA: **Functional genomic analysis of phagocytosis and identification of a *Drosophila* receptor for *E. coli***. *Nature* 2002, **416**:644-648.
An RNA-interference-based screen conducted in *Drosophila* S2 cells that identified a surface receptor for *Escherichia coli* as well as 34 gene products involved in phagocytosis. Several of these proteins have been previously implicated in vesicular transport, signaling, actin cytoskeletal remodeling or macrophage (haemocyte) development. This paper establishes *Drosophila* as a valuable model organism for phagocytosis.

40. Perry DG, Daugherty GL, Martin WJ II: **Clathrin-coated-pit-associated proteins are required for alveolar macrophage phagocytosis**. *J Immunol* 1999, **162**:380-386.

41. Hackam DJ, Botelho RJ, Sjolin C, Rotstein OD, Robinson JM, Schreiber AD, Grinstein S: **Indirect role for COPI in the completion of FCγ-receptor-mediated phagocytosis**. *J Biol Chem* 2001, **276**:18200-18208.

42. Gold ES, Morrissette NS, Underhill DM, Guo J, Bassetti M, Aderem A: **Amphiphysin IIm, a novel amphiphysin II isoform, is required for macrophage phagocytosis**. *Immunity* 2000, **12**:285-292.

43. van Dam EM, Stoorvogel W: **Dynamin-dependent transferrin receptor recycling by endosome-derived clathrin-coated vesicles**. *Mol Biol Cell* 2002, **13**:169-182.

44. • Di A, Nelson DJ, Bindokas V, Brown ME, Libunao F, Palfrey HC: **Dynamin regulates focal exocytosis in phagocytosing macrophages**. *Mol Biol Cell* 2003, **14**:2016-2028.
This paper uses the powerful capacitance measurements technique to show that dynamin controls exocytosis of membrane from internal stores to sites of phagocytosis in macrophages.

45. Damiani MT, Colombo MI: **Microfilaments and microtubules regulate recycling from phagosomes**. *Exp Cell Res* 2003, **289**:152-161.

46. Allen LA, Yang C, Pessin JE: **Rate and extent of phagocytosis in macrophages lacking VAMP3**. *J Leukoc Biol* 2002, **72**:217-221.

47. Bogdanovic A, Bennett N, Kieffer S, Louwagie M, Morio T, Garin J, Satre M, Bruckert F: **Syntaxin 7, syntaxin 8, Vti1 and VAMP7 (vesicle-associated membrane protein 7) form an active SNARE complex for early macropinocytic compartment fusion in *Dictyostelium discoideum***. *Biochem J* 2002, **368**:29-39.

48. McNew JA, Parlati F, Fukuda R, Johnston RJ, Paz K, Paumet F, Sollner TH, Rothman JE: **Compartmental specificity of cellular membrane fusion encoded in SNARE proteins**. *Nature* 2000, **407**:153-159.

49. Cox D, Lee DJ, Dale BM, Calafat J, Greenberg S: **A Rab11-containing rapidly recycling compartment in macrophages that promotes phagocytosis**. *Proc Natl Acad Sci USA* 2000, **97**:680-685.

50. Hickson GRX, Matheson J, Riggs B, Maier VH, Fielding AB, Prekeris R, Sullivan W, Barr FA, Gould GW: **Arfophilins are dual Arf/Rab11 binding proteins that regulate recycling endosome distribution and are related to *Drosophila* nuclear fallout**. *Mol Biol Cell* 2003, **14**:2908-2920.

51. Collins RF, Schreiber AD, Grinstein S, Trimble WS: **Syntaxins 13 and 7 function at distinct steps during phagocytosis**. *J Immunol* 2002, **169**:3250-3256.

52. Vieira OV, Bucci C, Harrison RE, Trimble WS, Lanzetti L, Gruenberg J, Schreiber AD, Stahl PD, Grinstein S: **Modulation of Rab5 and Rab7 recruitment to phagosomes by phosphatidylinositol 3-kinase**. *Mol Cell Biol* 2003, **23**:2501-2514.

53. Gotthardt D, Warnatz HJ, Henschel O, Bruckert F, Schleicher M, Soldati T: **High-resolution dissection of phagosome maturation reveals distinct membrane trafficking phases**. *Mol Biol Cell* 2002, **13**:3508-3520.

54. Garin J, Diez R, Kieffer S, Dermine JF, Duclos S, Gagnon E, Sadoul R, Rondeau C, Desjardins M: **The phagosome proteome: insight into phagosome functions**. *J Cell Biol* 2001, **152**:165-180.

55. Rupper A, Grove B, Cardelli J: **Rab7 regulates phagosome maturation in *Dictyostelium***. *J Cell Sci* 2001, **114**:2449-2460.

56. Harrison RE, Bucci C, Vieira OV, Schroer TA, Grinstein S: **Phagosomes fuse with late endosomes and/or lysosomes by extension of membrane protrusions along microtubules: role of Rab7 and RILP**. *Mol Cell Biol* 2003, **23**:6494-6506.

57. Ellson CD, Anderson KE, Morgan G, Chilvers ER, Lipp P, Stephens LR, Hawkins PT: **Phosphatidylinositol 3-phosphate is generated in phagosomal membranes**. *Curr Biol* 2001, **11**:1631-1635.

58. Vieira OV, Botelho RJ, Rameh L, Brachmann SM, Matsuo T, Davidson HW, Schreiber A, Backer JM, Cantley LC, Grinstein S: **Distinct roles of class I and class III phosphatidylinositol 3-kinases in phagosome formation and maturation**. *J Cell Biol* 2001, **155**:19-25.

59. Zerial M, McBride H: **Rab proteins as membrane organizers**. *Nat Rev Mol Cell Biol* 2001, **2**:107-117.

60. Defacque H, Bos E, Garvalov B, Barret C, Roy C, Mangeat P, Shin HW, Rybin V, Griffiths G: **Phosphoinositides regulate membrane-dependent actin assembly by latex bead phagosomes**. *Mol Biol Cell* 2002, **13**:1190-1202.

61. Kjeken R, Egeberg M, Habermann A, Kuehnel M, Peyron P, Floetenmeyer M, Walther P, Jahraus A, Defacque H, Kuznetsov SA *et al.*: **Fusion between phagosomes, early and late endosomes: a role for actin in fusion between late, but not early endocytic organelles**. *Mol Biol Cell* 2004, **15**:345-358.

62. Di A, Krupa B, Bindokas VP, Chen Y, Brown ME, Palfrey HC, Naren AP, Kirk KL, Nelson DJ: **Quantal release of free radicals during exocytosis of phagosomes**. *Nat Cell Biol* 2002, **4**:279-285.

63. Ramachandra L, Noss E, Boom WH, Harding CV: **Phagocytic processing of antigens for presentation by class II major histocompatibility complex molecules**. *Cell Microbiol* 1999, **1**:205-214.

64. Lehner PJ, Cresswell P: **Recent developments in MHC-class-I-mediated antigen presentation**. *Curr Opin Immunol* 2004, **16**:82-89.

65. •• Houde M, Bertholet S, Gagnon E, Brunet S, Goyette G, Laplante A, Princiotta MF, Thibault P, Sacks D, Desjardins M: **Phagosomes are competent organelles for antigen cross-presentation**. *Nature* 2003, **425**:402-406.

Together with [66••,67••], this paper shows that phagosomes function as cross-presenting organelles, competent for retrotranslocation of the phagocytosed antigens to the cytosol and loading of the generated peptides onto MHC_I molecules.

66. •• Guermonprez P, Saveanu L, Kleijmeer M, Davoust J, van Endert P, Amigorena S: **ER–phagosome fusion defines an MHC class I cross-presentation compartment in dendritic cells**. *Nature* 2003, **425**:397-402.

See annotation to [65••].

67. •• Ackerman AL, Kyritsis C, Tampe R, Cresswell P: **Early phagosomes in dendritic cells form a cellular compartment sufficient for cross presentation of exogenous antigens**. *Proc Natl Acad Sci U S A* 2003, **100**:12889-12894.

See annotation to [65••].

68. Lee WL, Harrison RE, Grinstein S: **Phagocytosis by neutrophils**. *Microbes Infect* 2003, **5**:1299-1306.

69. Brunet S, Thibault P, Gagnon E, Kearney P, Bergeron JJ, Desjardins M: **Organelle proteomics: looking at less to see more**. *Trends Cell Biol* 2003, **13**:629-638.

70. •• Blander JM, Medzhitov R: **Regulation of phagosome maturation by signals from Toll-like receptors**. *Science* 2004, **304**:1014-1018.

This work shows that engagement of Toll-like receptors (TLRs), known to induce pro-inflammatory signals, also influences the maturation of phagosomes via a cascade involving the MyD88 adaptor and p38 mitogen-activated protein kinase.