n higher organisms, phagocytosis is essential for eliminating infectious agents and for the scavenging of dead cells, whereas in lower unicellular organisms, phagocytosis is associated with food uptake. The phagocytic process can be divided into sequential events, starting with the recognition of the particle by dedicated receptors on the phagocytic cell surface. Among the best-characterized phagocytic receptors are opsonic receptors, and particularly Fcγ receptors (FcγRs). Clustering of FcγRs triggers a local and oriented polymerization of actin filaments that causes protrusion of the plasma membrane and wrapping of the particle within pseudopods. A contractile force is then generated to pull the particle into the cell, and the enclosed particle is finally degraded by lysosomal hydrolases after fusion of the phagosome with compartments of the endocytic pathway. Significant advances have recently been made in the molecular definition of the components of the phagocytic signal. Among several signalling molecules that are recruited to and are required at the site of phagocytosis, PtdInsP3 seems to be essential for pseudopod extension and phagosome closure. However, in the absence of precisely identified ligand(s) for phosphatidylinositol-3,4,5-trisphosphate (PtdInsP3; the product of PI(3)K) at the phagocytic site, it has been difficult to assign a definitive function to PI(3)K in phagocytosis. Work presented in this issue has now identified a recently discovered myosin, Myosin-X, as a downstream effector of PI(3)K in FcγR-signalling, suggesting new roles for PI(3)K and PtdInsP3 in phagocytosis.

FcγRs are characterized by the presence of pairs of tyrosine residues in the receptor (or its accessory γ chain) cytoplasmic region. After binding of a particle, receptor clustering facilitates phosphorylation of these tyrosine residues by Src family kinases(s), and brings Syk, a tyrosine kinase with two tandem src homology 2 (SH2) domains, to the phagocytic site. In turn, Syk recruits and activates PI(3)K (ref. 3). By transfecting a macrophage cell line with green fluorescent protein-tagged PH domains that bind PI(3)K products, Grinstein and coworkers have recently confirmed that PtdInsP3 rapidly accumulates at sites of phagocytosis and disappears after the phagosome has been sealed off from the plasma membrane. The evidence that PI(3)K activity was required for phagocytosis came from experiments using the PI(3)K inhibitors wortmannin (Wtn) and LY294002 (refs 6,7). Both drugs significantly reduced the ingestion of large particles (greater than or equal to 4 μm), whereas uptake of smaller particles was unaffected. A surprising finding of these studies was that, in contrast with its role in growth factor-induced actin cytoskeleton reorganization and membrane ruffling, PtdInsP3 production was not required for actin assembly during FcγR-mediated phagocytosis.

If limitation of pseudopod extension and inhibition of phagosome closure by Wtn/LY294002 was not the result of inhibition of cytoskeletal processes, it raises the question of what it could be caused by. Based on the observation that Wtn also abolished the spreading of macrophages on antibody-coated surfaces (the so-called ‘frustrated phagocytosis’ model), and the concomitant exocytosis of membrane from
intracellular pools, Greenberg and colleagues have suggested that PI(3)K could regulate the focal delivery of internal membranes to the plasma membrane, a process thought to contribute to pseudopod extension as a compensatory mechanism for the loss of membrane taking place during engulfment. According to this model, the severity of inhibition by Wt/Myo-VII mutants increases with particle size and hence, with the amount of membrane required to complete engulfment. This conclusion was also supported by evidence that indicated a requirement for components of the general membrane fusion machinery, such as soluble NSF attachment protein receptor (SNARE) proteins and the ATPase NSF in phagocytosis. More recently, other studies have examined the exocytosis of a green fluorescent protein (GFP)-tagged fusion of VAMP3, a SNARE protein that is localized to recycling early endosomes, which function as intermediates on the receptor recycling pathway. During FCγR-mediated phagocytosis, VAMP3-positive recycling endosomes fused with the plasma membrane in a polarized fashion, resulting in accumulation of GFP–VAMP3 at the phagocytic site (see Fig. 1). Wttn was found to inhibit the exocytosis of markers of recycling endosomes during phagocytosis. These observations argue that recycling endosomes may function as a pool of internal membrane that can be readily mobilized to the cell surface in a PI(3)K-dependent manner as a fundamental part of the process of pseudopod extension.

Other studies have suggested the possibility that PI(3)K may be involved in the generation of a contractile activity to complete phagosome closure. In earlier studies, forces produced during engulfment of yeast particles by phagocytes were measured. Using a micromechanical method, an alternate phase of engulfment and membrane extension occurring without contraction was observed, before a contraction phase that started abruptly and concomitant to particle ingestion. Furthermore, dumbled-shaped erythrocytes were observed in another study that examined macrophages attempting to ingest a single erythrocyte. The two bulbous ends of the erythrocyte, each enclosed in a phagosome within the two adjacent macrophages, were still connected by a thin membrane stalk. In the presence of Wt/Myo-VII mutants or butanediol monoxime (BDM, an inhibitor of myosins), constricted erythrocytes were absent. This argues that PI(3)K is involved in the generation of contractility restricted to the pseudopod margin and in the closure of the phagosome through a ‘purse-string-like’ mechanism. Several myosin isoforms (Myosin-Ic, -II, -Ixb and -V) that show a differential distribution at nascent phagosomes could conceivably control the generation of force during phagocytosis. The missing piece of the puzzle identified by Cox and colleagues is Myosin-X (ref. 4), a newly discovered myosin characterized by a tail domain containing three PH domains. Myosin-X is the founding member of a new class of unconventional myosins that have a similar motor domain to those of conventional myosins but lack unique structural tail domains that confer class-specific functions. Human Myosin-X is a 2058-amino-acid protein with head and tail domains separated by a region predicted to form a coiled coil, suggesting that Myosin-X heavy chains exist as dimers. The most unusual feature is the presence of three PH domains, the second of which interacts with PI(3)K products. The carboxy-terminal end contains a MYTH4 (myosin tail homology 4) and a FERM (4.1, ezrin, radixin, moesin) domain that are conserved in the tail region of other unconventional myosins. As expected, Myosin-X binds F-actin in an ATP-sensitive manner, and is enriched in a F-actin-rich phenotype, including lamellipodia and filopodia, in several cell types. In a previous issue, it was reported that Myosin-X accumulates at the tips of filopodia in agreement with the recent demonstration that Myosin-X is a motor that moves classically toward the barbed ends of actin filaments. Moreover, overexpression of Myosin-X resulted in a fivefold increase in the density of filopodia at the cell periphery. These findings strongly argue for a function of Myosin-X in processes that induce the protrusion of the plasma membrane. In this issue, Cox and colleagues show that macrophage Myosin-X accumulates at the phagocytic site with similar kinetics to F-actin, and importantly, this occurs in a PI(3)K-dependent manner (that is, recruitment of markers of the recycling pathway to the phagocytic site. Another possibility is that Myosin-X could be involved in generating the forces required for particle engulfment by pulling on the actin filament network that is present at the phagocytic site. Finally, the explanation that is most favoured by the authors is that by binding PtdInsP3, enriched membrane and simultaneously moving along actin filaments, Myosin-X lifts bulk plasma membrane in the direction of the barbed ends that face the outer margin of the forming phagosome. Coupled to this process, exocytosis of recycling endosomes provides the extra membrane required for pseudopod extension (see Fig. 1).

Over the past five years, components that contribute to various aspects of the phagocytic signal have been identified. The concept that directed actin polymerization drives the protrusion of the plasma membrane has been extremely fruitful. Along these lines, proteins such as Rho family GTPases and their effectors have been found to participate actively in actin dynamics during phagocytosis. However, another concept has also emerged borrowed from the related problem of cell motility. This view, initially suggested by M. Bretscher, proposed that the polarized insertion of membrane at the leading edge of a motile cell pushes it forward. The finding that Myosin-X functions in phagocytosis by linking PI(3)K and pseudopod extension,
and may couple the forward advance of the actin filament network to the pseudopodial movement of the plasma membrane, is important. This result brings together actin polymerization and membrane delivery to explain phagocytosis.

The ability to chemotax, that is, to sense and move in the direction of chemical signals, is a feature of a wide variety of eukaryotic cells. Chemotaxis is important for many biological responses, from the movement of leukocytes towards sites of infection or inflammation to the aggregation of Dicyostelium discoideum amoebae to form a multicellular organism. Recent work has firmly established the importance of the phosphatidylinositol 3-OH kinase (PI(3)K) pathway in mediating directional movement in response to chemotaxants. Insight into the mechanism that translates a shallow gradient of chemoattractant into cytoskeletal polarization and directional movement first came from work using Dicyostelium cells, and subsequently from studies with leukocytes and fibroblasts. These studies identified the importance of localized signalling by demonstrating that green fluorescent protein (GFP) fusions of a subfamily of pleckstrin homology (PH) domain-containing proteins, which specifically bind to the phosphoinositide products of PI(3)K, preferentially localized to the leading edge (see figure). Actin dynamics are necessary for the maintenance of the localized accumulation of PI(3)K activity downstream of PtdIns(3,4,5)P3 accumulation, is in turn necessary for the formation of a shallow extracellular gradient of chemoattractant into a steep intracellular secondary messenger gradient. In neutrophils, uniform stimulation with chemoattractant eventually results in spontaneous polarization. Wang et al. demonstrate that a membrane-permeable PtdIns(3,4,5)P3 complex can elicit the same response. Using a pharmacological approach, the authors go on to show that this response is dependent on endogenous PI(3)K activity and requires a Rho family GTPase activity. Their studies suggest a model for chemotaxis in which a directional chemotactic signal results in a small initial activation of PI(3)K, triggering a Rho GTPase-dependent feedback loop that amplifies the signal, contributing to the observed steep intracellular PtdIns(3,4,5)P3 gradient.

Weiner et al. provide further evidence that inhibition of PI(3)K activity impairs the ability to maintain stable pseudopodia, resulting in poor chemotactic fidelity. In addition, the authors provide compelling evidence that actin polymerization at the leading edge, which drives pseudopod extension and occurs through a positive feedback loop that amplifies the signal, is necessary for the maintenance of the localized accumulation of PtdIns(3,4,5)P3 at the leading edge (see figure). Actin dynamics as part of a positive feedback loop may provide neutrophils with the ability to spontaneously polarize in response to an initially diffuse stimulus and start moving, only later homing in on their target. Thus, the amplification of the response to a chemoattractant gradient by the combination of Rho GTPase and actin feedback loops provides an attractive mechanism for how an initial small response results in strong cell polarization and persistent chemotactic movement. What is unknown in this model are the mechanisms positioning the initial response that result in the eventual results in spontaneous polarization. Wang et al. demonstrate that a membrane-permeable PtdIns(3,4,5)P3 complex can elicit the same response. Using a pharmacological approach, the authors go on to show that this response is dependent on endogenous PI(3)K activity and requires a Rho family GTPase activity. Their studies suggest a model for chemotaxis in which a directional chemotactic signal results in a small initial activation of PI(3)K, triggering a Rho GTPase-dependent feedback loop that amplifies the signal, contributing to the observed steep intracellular PtdIns(3,4,5)P3 gradient.

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