

## Review

## Squeezing in a Meal: Myosin Functions in Phagocytosis

Sarah R. Barger,<sup>1</sup> Nils C. Gauthier,<sup>2</sup> and Mira Krendel<sup>1,\*</sup>

**Phagocytosis is a receptor-mediated, actin-dependent process of internalization of large extracellular particles, such as pathogens or apoptotic cells. Engulfment of phagocytic targets requires the activity of myosins, actin-dependent molecular motors, which perform a variety of functions at distinct steps during phagocytosis. By applying force to actin filaments, the plasma membrane, and intracellular proteins and organelles, myosins can generate contractility, directly regulate actin assembly to ensure proper phagocytic internalization, and translocate phagosomes or other cargo to appropriate cellular locations. Recent studies using engineered microenvironments and phagocytic targets have demonstrated how altering the actomyosin cytoskeleton affects phagocytic behavior. Here, we discuss how studies using genetic and biochemical manipulation of myosins, force measurement techniques, and live-cell imaging have advanced our understanding of how specific myosins function at individual steps of phagocytosis.**

## Introduction

Phagocytosis is an ancient biological process, utilized initially for nutrient uptake and now a critical activity for immune defense [1]. Professional phagocytes, including neutrophils, dendritic cells, and macrophages, regularly rid the body of pathogens, apoptotic cells, and cellular debris. These phagocytic targets are recognized by specific cell-surface receptors, which relay distinct downstream signals through protein and lipid kinases and phosphatases, small GTPases, and other signaling proteins [2]. Targets coated by plasma- or cell-derived components (opsonins) are recognized by opsonic receptors, including Fc receptors (FcRs) that bind the conserved domain of immunoglobulins and complement receptors (CRs), which respond to targets coated in the complement derivative iC3b [1]. Non-opsonic receptors (receptors binding to specific motifs on phagocytic targets) include Dec-1 receptors that recognize fungal  $\beta$ -glucan and scavenger receptors that interact with both apoptotic and microbial ligands [1,3]. For internalization, phagocytic receptors can work in concert, with others serving to modulate the phagocytic response [4]. Despite the diversity of phagocytic receptors recognizing various targets, the process of target internalization always requires the actin cytoskeleton [5–9].

Phagocytosis by FcRs is classically divided into multiple steps. First, phagocytes search for targets using membrane protrusions, such as filopodia or ruffles, generated by actin polymerization [10]. This active process of probing the cell's microenvironment can be stimulated, or primed, by soluble molecules such as TLR ligands and growth factors, which increase actin-dependent ruffling [11]. Once contact is made, phagocytic receptors cluster resulting in a more stable adhesion [12]. Downstream signaling from engaged receptors leads to robust actin polymerization that deforms the plasma membrane, causing the cell to encircle the target in a structure known as the phagocytic cup [13]. Closure of the phagocytic cup produces a sealed membrane-bound organelle called the phagosome, which is trafficked along microtubules further into the cell. Fusion of the phagosome with endosomal vesicles and later lysosomes is required for pathogen degradation and antigen presentation [1,14].

Myosins, actin-dependent molecular motors (Box 1), work to apply direct force on actin filaments, but their mechanical role in phagocytosis is not fully understood. This is due partially to the multitude of myosin isoforms that are expressed in phagocytes (Figure 1), as well as to the relatively fast and complex multistep nature of phagocytosis. Myosins can regulate actin assembly, actin filament crosslinking and rearrangement, actin-dependent membrane deformation, and protein localization. In this review, we examine our current understanding of the role of myosins in the various steps of

## Highlights

Phagocytosis in both mammalian and nonmammalian cells requires F-actin and involves multiple myosin isoforms.

Specific steps in phagocytosis rely on the activity of specific myosin isoforms.

Phagocytes encounter targets with varying physical characteristics, which may affect the activity and distribution of the involved myosins.

The physiological setting of a phagocyte affects its actomyosin cytoskeleton and in turn its phagocytic behavior.

<sup>1</sup>Cell and Developmental Biology Department, State University of New York Upstate Medical University, Syracuse, NY, USA

<sup>2</sup>IFOM, FIRC Institute of Molecular Oncology, Milan, Italy

\*Correspondence: [krendelm@upstate.edu](mailto:krendelm@upstate.edu)



**Box 1. Myosins Implicated in Phagocytosis**

Myosin motors comprise heavy chains, which are responsible for myosin motor activity, and light chains, which are important for myosin force production and regulation [104]. Myosin heavy chains come in a wide variety of isoforms that form the myosin protein superfamily (see Figure 1 in main text). Myosin light chains are typically calmodulins or calmodulin-like calcium-binding proteins, with some myosins having dedicated light chains [105]. While all myosin heavy chains contain an N-terminal motor domain and a neck region that binds light chains, the tail domains of myosins are highly divergent. These diverse tails account for the ability of myosins to localize to specific subcellular regions and interact with cargo. Furthermore, while the overall structure and function of the myosin motor domain is conserved, different myosin isoforms are characterized by distinct mechanochemical properties, such as the rate of ATP hydrolysis, processivity (ability to take multiple steps along an actin filament), the duty ratio (fraction of the ATPase cycle spent strongly attached to actin), and the velocity of movement along actin [106]. Based on the sequence homology of the motor and structural similarities of the tail domains, the myosin superfamily is divided into distinct myosin classes. Evidence for a myosin's contribution in phagocytosis comes primarily from: (i) localization studies, including those examining recruitment signals and motifs that guide myosin enrichment during phagocytosis; (ii) chemical inhibition of myosin activity; (iii) expression of dominant-negative constructs; and (iv) studies of myosin-null cells.

phagocytosis, giving special attention to the mechanochemical features of each myosin and how they function in the overall biomechanics of the process. Furthermore, we discuss how the use of force measurements and engineered phagocytic targets and microenvironments has offered new insights into myosin activity during this process.

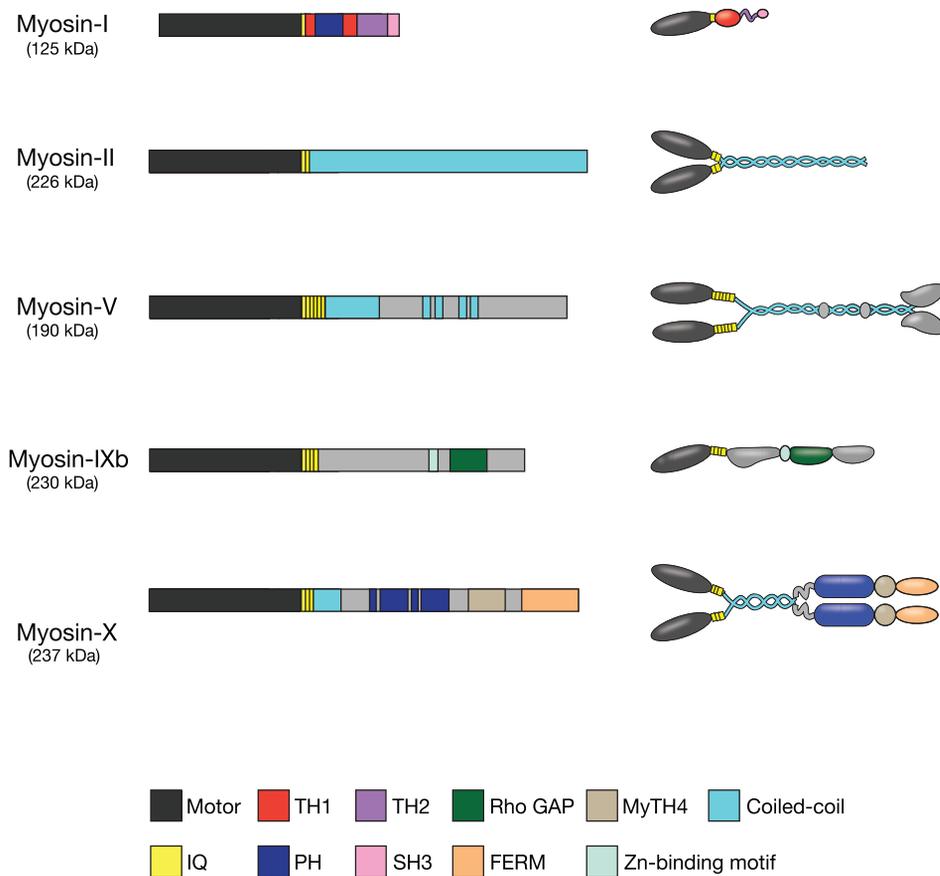
**Target Capture Using Myosin-X-Guided Filopodia**

Phagocytes capture their targets through actin-based membrane ruffles or filopodia, which can act as cellular tentacles, extending outward to bind a target and retracting back to pull the target to the cell surface [10,15]. Myosin-X is ubiquitously expressed among all immune cells and is well known for its striking localization at filopodial tips [16,17]. It is a double-headed processive motor with a tail that includes a myosin tail homology 4 domain and band 4.1/ezrin/radixin/moesin domain (MyTH4-FERM) [17,18]. Myosin-X in macrophages was initially observed to be recruited to the phagocytic cup and exogenous expression of the myosin-X tail domain as a dominant-negative construct reduced phagocytosis of antibody-coated erythrocytes by ~80% [19]. Myosin-X tail specifically reduced the uptake of large but not small IgG-coated beads, suggesting that this motor had a specific role in pseudopod extension during phagocytic internalization. However, two new studies now show that myosin-X knockout (KO) macrophages ingest zymosan (yeast particles) and IgG-coated beads or cells at a rate indistinguishable from wild-type cells [20,21]. Instead, myosin-X-null macrophages seem to form fewer filopodia in response to LPS or *Escherichia coli* [20]. Thus, despite localization to the phagocytic cup, myosin-X appears to be dispensable for actin dynamics during internalization and is likely to contribute to the earliest step of phagocytosis, filopodia initiation and maintenance to capture phagocytic targets (Figure 2).

**Assembling the Phagocytic Cup: Roles for Myosin-I and Myosin-IX**

Once a phagocytic target contacts the surface of the cell, receptor clustering and downstream signaling initiate the formation of the phagocytic cup that surrounds the target. The assembly of this actin-based structure is likely to involve at least two myosin classes: myosin-I and myosin-IX (Figure 2).

Myosin-I's are small, monomeric motors that represent one of the largest groups in the myosin superfamily. Myosin-I's contain a single heavy chain with a tail domain divided into three conserved regions abbreviated as TH1, TH2, and TH3 for tail homology 1, 2, and 3. The TH1 domain participates in membrane binding, the TH2 domain contains an ATP-insensitive actin binding site, and the TH3 domain is a Src homology 3 (SH3) domain known to participate in protein-protein interactions [22]. Myosin-I's that contain only the TH1 domain are called 'short-tailed' while those that contain all three tail domains are known as 'amoeboid' or 'long-tailed' [23].



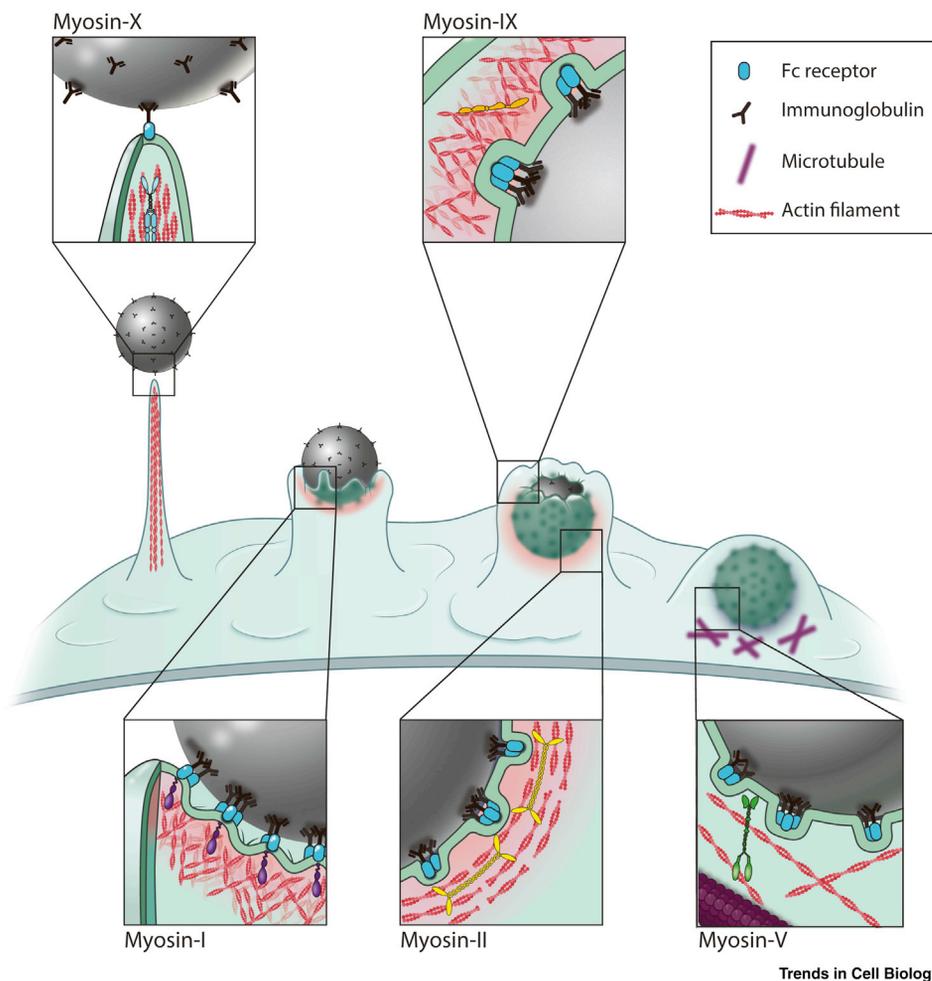
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### Figure 1. Domain Organization and Overall Structural Features of Myosin Isoforms Involved in Phagocytosis.

Each myosin heavy chain comprises an N-terminal motor domain, a neck domain that binds light chains, and a tail domain that can include coiled-coil motifs to promote dimerization (in myosin-II, -V, and -X), membrane-binding Pleckstrin homology (PH) domains (in myosin-I and -X), or protein interaction motifs that promote cargo binding specificity. Abbreviations: TH1, tail homology 1; TH2, tail homology 2; IQ, light chain-binding IQ motif; SH3, Src Homology 3; MyTH4, myosin tail homology 4; GAP, GTPase-accelerating protein. Redrawn from [112].

The participation of myosin-I<sub>s</sub> in phagocytosis from single-celled organisms to mammalian phagocytes makes this class of myosins an evolutionarily conserved group of phagocytic motor proteins. Myosin-I<sub>s</sub> in *Acanthamoeba castellanii*, a free-living soil amoeba, and *Entamoeba histolytica*, a parasitic protozoan that depends on phagocytosis of human cells, are strongly recruited to phagocytic cups [24,25]. Of the seven myosin-I<sub>s</sub> in the slime mold *Dictyostelium discoideum*, both short-tailed (Myo1E) and long-tailed (Myo1B and Myo1C) isoforms contribute to phagocytosis, with short- and long-tailed myosins likely to play nonoverlapping roles in this process [26–30]. Myo1K, a structurally divergent myosin-I, also plays a role in phagocytosis, with Myo1K<sup>−</sup> cells ingesting ~30% fewer yeast cells than wild-type *Dictyostelium* [31]. Thus, while myosin-I<sub>s</sub> are not absolutely essential for phagocytosis, they are required for efficient target uptake and multiple isoforms often need to be depleted to observe an effect on internalization. An unanswered question is why only some members of this myosin class localize to phagocytic cups and, among those, how certain isoforms (e.g., long-tailed vs short-tailed) perform distinct, nonoverlapping functions.

The mammalian genome encodes eight myosin-I isoforms (Myo1a–h; this nomenclature is not related to that of *Dictyostelium*), with immune cells containing varying combinations of short-tailed



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### Figure 2. Schematic of the Sequential Steps of FcR-Mediated Phagocytosis and the Specific Myosin Isoforms Involved.

Myosin-X-mediated filopodia function in phagocytic target search and capture. Myosin-I (Myo1e/f) and -IX (Myo9b) enable productive actin assembly in the phagocytic cup, with myosin-II-generated contractility at the base. Myosin-V (Myo5a) assists in anchoring the phagosome once internalized.

(Myo1c and Myo1g) and long-tailed (Myo1e and Myo1f) myosin-IIs [32]. In macrophages, Myo1g, Myo1e, and Myo1f are recruited to the phagocytic cup during uptake of IgG-coated targets [33–37], but closer examination reveals slightly different localizations. Myo1g is enriched at the membrane along the sides of the phagocytic cup [37], while Myo1e and Myo1f localize at the leading edge of the phagocytic cup as well as at distinct adhesion sites within the cup [36]. The ability of myosin-IIs to bind both actin and membrane and therefore maintain membrane–cortex adhesion has been shown to be important for the regulation of membrane tension [38], which in turn is important for phagocytosis [39]. Reduced membrane–cytoskeletal adhesion during phagocytosis, as observed in Myo1e and Myo1f double-KO macrophages, correlates with the assembly of an excessively dense actin meshwork in the phagocytic cup and slower phagocytic internalization [36]. Recently, these phagocytic adhesions have been likened to podosomes [40], specialized actin-based adhesive structures that are capable of degrading the extracellular matrix [41]. Moving forward, it will be interesting to determine whether there are mechanistic parallels between the role of myosins in the regulation of podosome dynamics [42,43] and their contribution to actin dynamics in the phagocytic cup.

The second myosin class believed to participate in phagocytic cup assembly is myosin-IX, specifically Myo9b, which has been detected at both phagocytic cups and isolated phagosomes [44,45]. A genome-wide CRISPR screen using a human macrophage cell line showed that KO of Myo9b inhibited the phagocytosis of positively and negatively charged beads of various sizes, as well as IgG-opsonized red blood cells [46]. In dendritic cells derived from the bone marrow of Myo9b KO mice, however, internalization of *E. coli* was unaffected [47]. Myosin-IX is a unique single-headed processive motor capable of remaining attached to an actin filament throughout the ATPase cycle by an extended loop in the motor domain [48]. It may be able to act as an actin crosslinker, since purified Myo9a mixed with actin creates an evenly spaced actin lattice structure [49]. In addition, the myosin-IX tail contains a RhoGAP domain, which inactivates specifically the small GTPase Rho [50]. As a result, Myo9b KO macrophages exhibit significantly higher levels of activated RhoA and cannot form membrane protrusions, maintaining a rounded-up appearance [51]. These distinctive motor properties controlling a potential signaling hub suggest a fascinating role for Myo9b in phagocytosis. Given the need for F-actin to remain dynamic during internalization, Rho family GTPases continuously cycle between 'on' and 'off' states throughout phagocytosis with the help of activating GEFs and deactivating GAPs [52,53]. Classically, RhoA GTPase is known to be important for CR-mediated phagocytosis but dispensable for phagocytosis through FcR [54]. However, Myo9b is clearly recruited to the phagocytic cup around IgG-coated targets [53]. This finding suggests either that Myo9b is not functioning in Rho inactivation at these sites or that the activity of small GTPases during phagocytic cup assembly is more complex than we currently understand.

### Closing the Phagocytic Cup and Myosin-II-Generated Contractility

A role for myosin-II in phagocytosis has been suspected since its detection at sites of ingestion by early immunofluorescence studies of macrophages [55]. Mammalian cells contain three non-muscle myosin-II isoforms: non-muscle myosin-2A (NM2A), NM2B, and NM2C, encoded by the *MYH9*, *MYH10*, and *MYH14* genes in humans. NM2A is the dominant myosin-II isoform expressed in immune cells [32,56]. Like skeletal muscle myosin-II, this myosin functions as a heterohexamer comprising a dimer of heavy chains and four associated light chains: two essential light chains (ELCs) and two regulatory light chains (RLCs) [57]. These heterohexamers further assemble into filaments or minifilaments, bipolar structures (with myosin motor domains at each end of the filament) that crosslink and contract actin filaments by promoting the sliding of actin filaments relative to each other. Regulation of NM2A is accomplished via reversible phosphorylation of the RLC by multiple kinases including myosin light-chain kinase (MLCK) and Rho-associated coiled-coil-containing kinase (ROCK) as well as via regulation of the activity of myosin light-chain phosphatase by ROCK and other regulators [58]. RLC phosphorylation not only increases the ATPase activity of the motor, but also facilitates the assembly of myosin-II filaments [58,59]. The contribution of myosin-II in phagocytosis has been assessed using chemical inhibition of upstream myosin kinases as well as the motor activity itself and the results differ, leading to uncertainty on whether myosin-II activity is critical for phagocytosis. However, with the ability of bipolar myosin filaments to contract the F-actin network, NM2A may contribute to particle internalization via constriction of the phagocytic cup (Figure 2). On closer examination, differing experimental results may depend on cell type, the type of phagocytosis examined (FcR- or CR-mediated), the timing of experiments (duration of incubation prior to evaluation of phagocytic efficiency), and the target and concentration of the inhibitor used (with different inhibitors affecting myosin ATPase activity either directly or indirectly via inhibition of MLCK or Rho kinase).

Inhibition of MLCK in neutrophils using ML-7 halts phagocytic internalization [60] while inhibition of MLCK in macrophages produces a less dramatic effect [61,62,64]. However, in macrophages treated with ML-7, phagocytic cups are splayed open and appear not to squeeze IgG-coated red blood cells [62]. The NM2A expression level positively correlated with phagocytic ability using a macrophage cell line ingesting sheep red blood cells [63]. Direct inhibition of myosin-II ATPase activity using the drug blebbistatin has been shown to reduce phagocytosis to varying degrees, depending on the length of the experiment and the size of the phagocytic particles [63–65]. Together, these observations suggest that, at least in some settings, myosin-II activity contributes to timely phagocytic internalization and may promote close apposition between the phagocytic cup and the surface of the target. In a 2D

approximation of phagocytosis known as frustrated phagocytosis, macrophages treated with blebbistatin exert significantly reduced contractile forces, as measured by traction force microscopy [66]. It is also possible that myosin-II, like myosin-I and myosin-IX, plays a role in phagocytic cup assembly, although the evidence is again somewhat conflicting. In J774.A1 macrophages, myosin activity is dispensable for phagocytic cup assembly during FcR-mediated phagocytosis but critical for CR-mediated phagocytosis [67], while in RAW macrophages and primary bone marrow-derived macrophages, actin accumulation is drastically reduced by ML-7 and blebbistatin treatment during FcR-mediated phagocytosis [64].

Overall, it appears likely that myosin-II generates contractile force in the phagocytic cup during internalization to promote efficient closure. While inhibitor studies indicate that myosin-II motor activity is required for efficient target ingestion, the precise timing and location of NM2A's contractile activity remain to be elucidated. It is also unknown how actin filaments in the phagocytic cup are arranged for such contractile force. Typically, subcellular actin assemblies subject to myosin-II-mediated contraction comprise F-actin of opposing polarity with varying degrees of actin and myosin-II filament alignment [68]. Whether phagocytic cups include similar antiparallel arrangements of actin filaments for myosin-II activity is unclear, although, using STED microscopy, concentric rings of F-actin, consistent with an ordered and aligned arrangement of actin filaments, have been observed in phagocytic cups of dendritic cells engulfing yeast [69]. However, for myosin-II to act productively on such filaments, these F-actin bundles would need to be immobilized – anchored at the membrane or crosslinked to each other. One of the candidates for this role is  $\alpha$ -actinin, an actin-crosslinking protein that has been localized to the phagocytic cup [70,71] and that helps to anchor actin filaments to transmembrane proteins at Z-disks or dense bodies of muscle myofibers and at focal adhesions [72]. In the future, it will be important to determine where and in what direction myosin-II-generated force is applied in the cup (e.g., does it result in tangential contraction along the constricting edge of the cup or uniform radial compression throughout the entire actin-rich cup?). Reexamination of myosin-II recruitment, localization, and activation using improved imaging tools and biosensors providing high spatial and temporal precision [73,74] may help to resolve some of the controversies regarding the functions of this myosin class in phagocytosis.

### Transporting the Phagosome with Myosin-V

Closure of the phagocytic cup creates a membrane-bound organelle known as the phagosome, which is shuttled further into the cell for processing and degradation [1,14]. Retrograde transport of the phagosome is known to occur along microtubule tracks [75], but it also involves myosin-V, a large, two-headed myosin motor. In macrophages from *dilute-lethal* (*Myo5a*-null) mice, following internalization, phagosomes move toward the cell center twice as fast as in wild-type cells, rapidly accumulating in the perinuclear region [76]. As a processive motor with a  $\sim 36$ -nm step size, *Myo5a* is kinetically adaptable for the transport of large vesicular cargos including organelles [77–80]. Since most actin filaments in mammalian cells are relatively short, the likely roles for *Myo5a* are: (i) participation in short-range/local trafficking of organelles following their long-range delivery along microtubules; or (ii) competition with faster microtubule-dependent transport by serving as an anchor to maintain organelle distribution in specific cellular regions. The study using *dilute-lethal* macrophages [76] suggests that *Myo5a* competes with dynein-mediated centripetal transport on microtubules, a feature that has previously been observed during transport of melanosomes in *dilute-lethal* melanocytes [81]. The physiological significance of this *Myo5a*-dependent delay in centripetal movement with frequent stops and reversals of phagosomes is still unknown, although it may be important to promote phagosome interactions with specific organelles at the cell periphery. Furthermore, *Myo5a* may play a role in vesicle transport during internalization, as increased membrane surface area is required for internalization of large targets.

In mammals, the myosin-V tail is linked to cargo via adaptor complexes and myosin-V motor activity is tightly regulated by adaptor binding, which triggers the unfolding of the autoinhibited protein to bind actin [82]. While the adaptor proteins that link *Myo5a* to phagosomes remain unknown, a yeast two-hybrid screen has shown that *Myo5a* interacts with a wide variety of Rab proteins [83], many of

**Box 2. Force Measurement Techniques in Studies of Phagocytosis**

Precise measurements of cellular forces during phagocytosis, along with the detection of concomitant changes in myosin activity and localization, are important in deciphering the contribution of myosin to phagocytic uptake. Specific types of forces associated with phagocytosis include pulling or pushing forces exerted on the target by the phagocyte, squeezing force generated by the contraction of the phagocytic cup, and changes in the cortical tension of the phagocyte during formation and closure of the phagocytic cup. These varied forces can be measured using several techniques. An optical trap holding an antibody-coated bead was used to measure the retraction (pulling) force of capturing filopodia, which ranged from <1 pN to >15 pN in macrophages and was not affected by knockdown of Myo5a, KO of myosin-VI, or inhibition of myosin-II [107]. Recent work combining the 2D frustrated phagocytosis assay with traction force microscopy has enabled assessment of the contractile forces exerted by the 'closing' phagocytic cup, which has been shown to be dependent on myosin-II [66] and not myosin-I [36]. Attaching an antibody-coated bead to an AFM cantilever is another technique recently used to probe the forces generated during phagocytic cup formation [108]. Micropipette aspiration has been used to measure changes in cortical tension during neutrophil phagocytosis of antibody-coated beads [109,110]. This has led to a model whereby phagocytosis proceeds through the dual action of the cytoskeletal pressure forcing membrane protrusion at the tips of the phagocytic cup and strong membrane–cytoskeleton adhesion at the target surface [109,110]. Studies using the tether pulling assay by optical tweezers similarly show that, as a cell stretches to engulf a large target, membrane tension increases with enhanced membrane–cytoskeletal adhesion [36,39]. A new technique [111] involving easily derivatized hydrogel microparticles, in which particle deformation can be used to accurately measure and localize subcellular forces during phagocytic internalization, will undoubtedly help in dissecting how each myosin is powering ingestion.

which are detected on phagosomes [84]. Thus, some of these Rabs may serve as regulating adaptors of myosin–phagosome complexes.

**Concluding Remarks**

The majority of myosin phagocytosis studies to date have been performed on cells *in vitro*. However, phagocytes reside in tissues with distinct physical characteristics and encounter phagocytic targets with various geometrical, mechanical, and chemical properties and the process of phagocytosis is affected by both cell environment and target properties. Since the actin cytoskeleton is influenced by substrate stiffness and geometry, myosin activity during phagocytosis is also likely to be modulated by the cell microenvironment. Engineered substrates and other approaches have been used to test how the cell microenvironment, including substrate stiffness, cell confinement, and extracellular pressure, affects phagocytosis [85–87]. These studies show that phagocytosis is stimulated by stiffer substrates but inhibited by spatial confinement [85,86]. Whether other physical features of the microenvironment, such as surface topography or interstitial flow, affect phagocytosis remains to be determined.

Classic targets used to study phagocytosis include both soft sheep red blood cells and stiff polystyrene beads, the latter being used for their easy coating, uniform shape, and availability in a variety of sizes. However, these polystyrene targets are orders of magnitude more rigid than anything a phagocyte will encounter *in vivo*. Interestingly, phagocytes have particular preferences when it comes to target size, shape, rigidity, and surface charge, which have been well studied using targets with more varied properties [88–97]. It is highly likely that the activity and utilization of the various myosin isoforms in phagocytes are influenced by the target's size, shape, rigidity, and surface properties. In one such example of myosin regulation by the mechanical properties of the target, artificial stiffening of red blood cells using glutaraldehyde pretreatment promoted the activation of NM2A in macrophages and enhanced FcR-mediated phagocytosis [98], perhaps supporting a system for the selective removal of aging red blood cells *in vivo*. Conversely, mechanosensitive regulation of CR-mediated phagocytosis appears to be independent of myosin-II [99]; thus, different types of phagocytic receptors may engage distinct mechanosensitive pathways (myosin dependent or independent).

Furthermore, mechanical cues from the target may directly affect the myosin mechanochemical cycle, such as ATP binding, ADP release, and detachment from the actin filament, which can be affected by the force applied to myosin [100]. The effects of force on myosin–actin binding can range from catch-bond-like behavior (detachment rate decreasing under load) to slip-bond behavior (detachment rate increasing under load) to power stroke reversal and even reverse sliding of myosin toward the minus end of the actin filament under load [100–102]. Such force-sensitive actomyosin interactions could partially account for the sensitivity of phagocytosis to the mechanical properties of the phagocytic target. For example, interactions with stiff targets could result in elevated recruitment of myosins that exhibit catch-bond-like response to load. This recruitment of myosin motors may in turn increase the rigidity of actomyosin networks, creating a positive feedback loop in response to target stiffness. Last, myosin activity may also be influenced by the shape of the target, given that myosin-II has been shown to preferentially associate with regions of low or negative membrane curvature [103].

As techniques that allow detailed dissection of the mechanosensitivity of phagocytosis and force production continue to be developed (Box 2), future work in this area is likely to shed light on the interplay between the properties of phagocytic targets and myosin recruitment, activation, and functions. Examination of the F-actin ultrastructure in the phagocytic cup using super-resolution microscopy or cryo-EM tomography will also be important since different myosin classes are characterized by a preference for a specific type of actin network (e.g., branched actin for myosin-I, antiparallel bundles for myosin-II). We encourage future research (see Outstanding Questions) to make use of bioengineered substrates to better examine phagocytosis in a more physiological setting.

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## Outstanding Questions

How does myosin activity alter the F-actin organization of the phagocytic cup?

Are myosins transporting any cargo during specific steps of phagocytosis?

Which steps during phagocytic internalization require contractility, what is the geometry/directionality/magnitude of the contractile forces, and which myosin isoforms are involved in force production?

How are specific myosin isoforms temporally and spatially regulated during different forms of phagocytosis?

Are myosins further regulated by external stimuli or by the physical characteristics of the phagocytic target?

How do myosins interact with the internalized phagosome? Are there specific phagosome adaptors used to anchor myosins?

How do the physical characteristics of the phagocyte’s environment *in vivo* affect myosin activity during phagocytosis?

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