Investigating Forces for Uptake and Cup Closure – The Role of Myosins in Phagocytosis

Anna E. Dart, Sylvain Tollis & Robert G. Endres

CISBIC, Imperial College, London, United Kingdom

Summary

Phagocytosis is initiated by the engagement of phagocyte surface receptors by a particulate target, which triggers the recruitment and activation of a variety of signalling molecules, culminating in the local reorganisation of the actin cytoskeleton and internalisation of the bound particle. The most extensively characterised phagocytic pathway to date stems from the Fcγ opsonin receptor. Ligation of the FcγR by an IgG-opsonised particle stimulates pseudopodial extensions of the cell membrane that surround and eventually enclose the particle. It is firmly established that dynamic actin polymerisation is essential for this process; however, it is less well understood how the localised contractile activity which constricts the margins of forming phagosomes coordinates with the actin cytoskeleton to extend pseudopods and close the phagocytic cup (Swanson et al., 1999). Several different motor proteins have been implicated in mammalian phagocytosis including members of the class I myosins, myosin IIa, myosin X and myosin Va (reviewed in Araki et al., 2006).

Myosins bind actin filaments and generate mechanical force by hydrolysing ATP. In general, their structure consists of an N-terminal head or motor domain enclosing the ATP- and actin-binding sites; a neck region containing repeats of a light chain-binding region termed the IQ motif; and a C-terminal tail domain. The class I myosins have described roles in a wide range of cellular events including membrane trafficking and formation of membrane protrusions (Coluccio, 2008). Interesting to this study, myosin IG has recently been shown to be a haematopoietic cell-specific myosin that regulates cell elasticity (Olety et al., 2010).

Herein we propose that the class I myosin, myosin IG is recruited to phagocytic cups upon ligation of the FcγR. Our data suggest that its localisation at the phagocytic cup is controlled by the activity of PI3K and that two conserved basic residues present in a PH-like domain of the tail are necessary for the functioning of myosin IG in internalisation of IgG-coated particles.

Necessity for a myosin-based force generation during FcγR-mediated phagocytosis

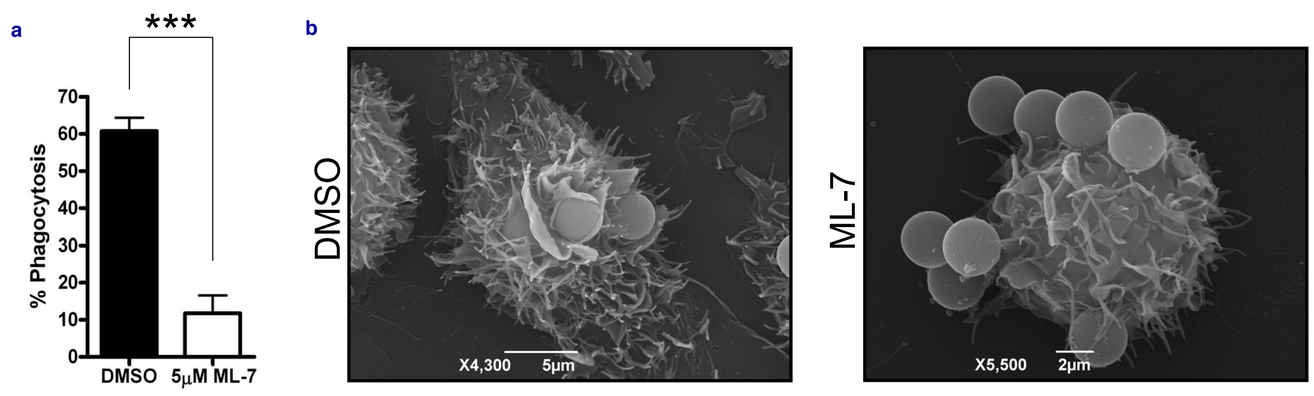


Figure 1: J774A.1 macrophages were pre-incubated with 5 μM of the MLCK inhibitor ML-7 or the DMSO vehicle before being challenged with IgG-opsonised beads for 15 minutes at 37˚C in the presence of the inhibitor. a) % phagocytosis, defined as the proportion of total bound IgG-beads that were internalised from 100 macrophages, was scored by differential Alexa fluor labelling pre- and post-permeabilisation. Results are the mean ± S.E.M of triplicate experiments. ✱✱✱, p<0.001. b) Characteristic SEM images of uptake via the FcγR in macrophages treated with DMSO or the drug. c) J774A.1 macrophages were challenged with IgG-beads for 15 minutes and then labelled with an anti-myoIIA antibody (Cell Signaling) and WGA to detect membrane. Arrow denotes a bead that is myosin IIA-positive and co-localises with the membrane marker. Scale bar, 10 μm.

Myosin IE and Myosin IG are recruited progressively to the phagocytic cup following FcγR ligation

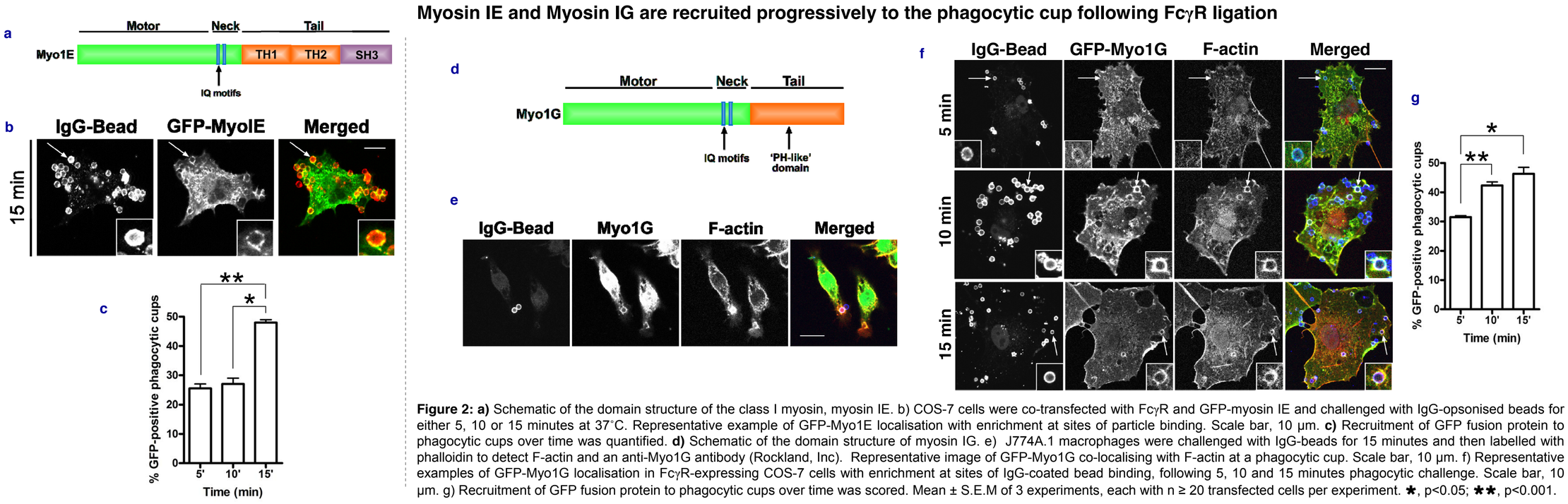


Figure 2: a) Schematic of the domain structure of the class I myosin, myosin IE. b) COS-7 cells were co-transfected with FcγR and GFP-myosin IE and challenged with IgG-opsonised beads for either 5, 10 or 15 minutes at 37˚C. Representative example of GFP-Myo1E localisation with enrichment at sites of particle binding. Scale bar, 10 μm. **c)** Recruitment of GFP fusion protein to phagocytic cups over time was quantified. **d)** Schematic of the domain structure of myosin IG. e) J774A.1 macrophages were challenged with IgG-beads for 15 minutes and then labelled with phalloidin to detect F-actin and an anti-Myo1G antibody (Rockland, Inc). Representative image of GFP-Myo1G co-localising with F-actin at a phagocytic cup. Scale bar, 10 μm. f) Representative examples of GFP-Myo1G localisation in FcγR-expressing COS-7 cells with enrichment at sites of IgG-coated bead binding, following 5, 10 and 15 minutes phagocytic challenge. Scale bar, 10 μm. g) Recruitment of GFP fusion protein to phagocytic cups over time was scored. Mean ± S.E.M of 3 experiments, each with n ≥ 20 transfected cells per experiment. ✱, p<0.05; ✱✱, p<0.001.

Myosin IG localisation at phagocytic cups is dependent on PI3K

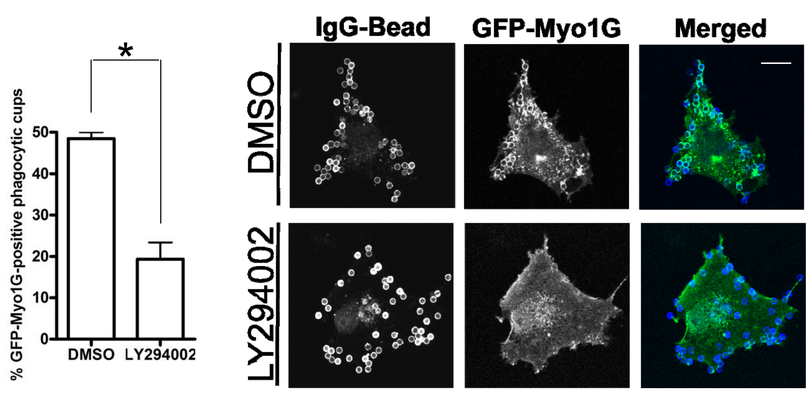


Figure 3: J774A.1 macrophages were pre-treated with 50 μM of the PI3K inhibitor LY294002 or the DMSO vehicle and then incubated with IgG-opsonised beads for 15 minutes at 37˚C in the presence of this drug. The recruitment of GFP-Myo1G to phagocytic cups under these conditions was quantified as % positive phagocytic cups. ✱, p<0.05. Representative confocal images of GFP-Myo1G localisation in FcγR-expressing COS-7 cells that have been incubated in either DMSO or LY294002. Scale bar, 10 μm.

The PH-like domain in Myosin IG is required for FcγR-mediated phagocytosis

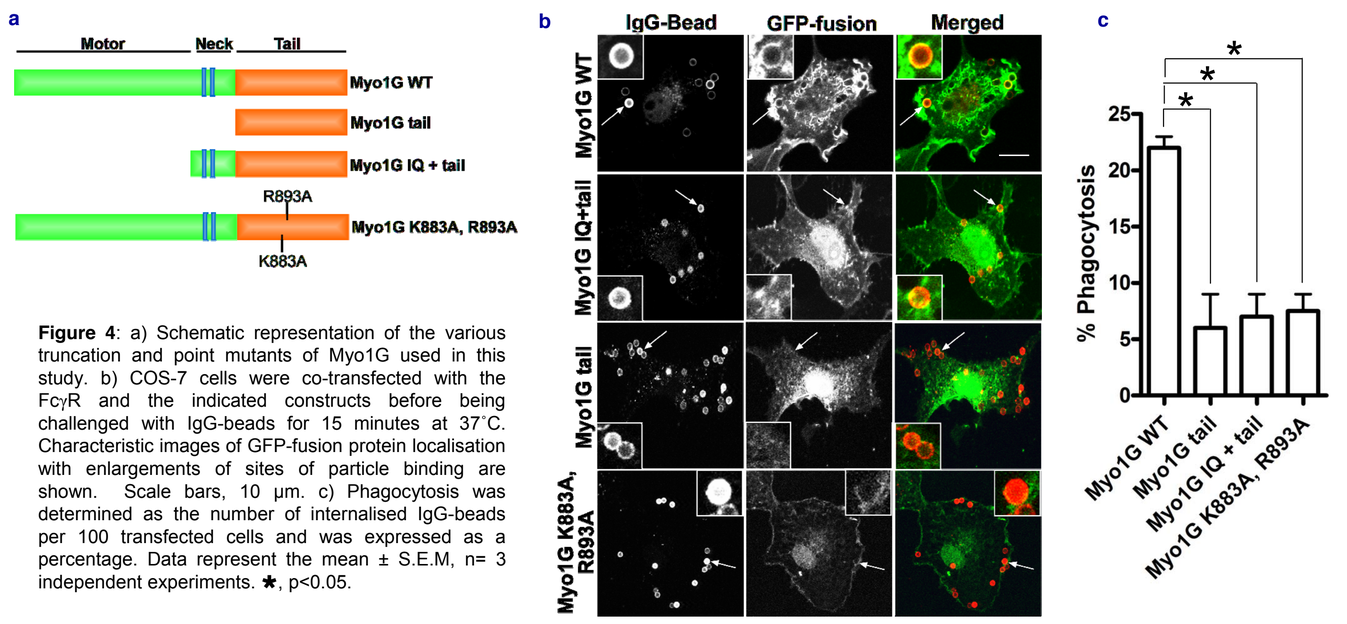


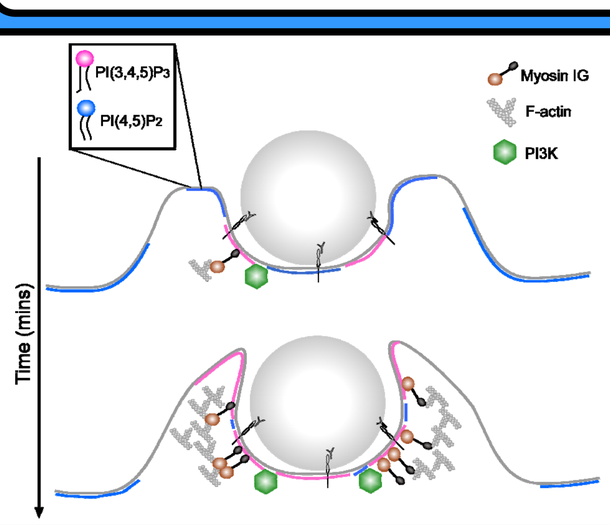
Figure 4: a) Schematic representation of the various truncation and point mutants of Myo1G used in this study. b) COS-7 cells were co-transfected with the FcγR and the indicated constructs before being challenged with IgG-beads for 15 minutes at 37˚C. Characteristic images of GFP-fusion protein localisation with enlargements of sites of particle binding are shown. Scale bars, 10 μm. c) Phagocytosis was determined as the number of internalised IgG-beads per 100 transfected cells and was expressed as a percentage. Data represent the mean ± S.E.M, n= 3 independent experiments. ✱, p<0.05.

Conclusions

We have shown that:

- Myosin function is important for FcγR phagocytic cup formation.
- Two unconventional class I myosins, myosins IE and IG are enriched at FcγR phagocytic cups. Their localisation coincides with the appearance of F-actin, peaking 15 minutes after IgG-bead binding.
- Myosin IG recruitment to the FcγR phagocytic cup is dependent on PI3K activity.
- The motor domain and the conserved basic residues in the PH-like domain of the tail of myosin IG are important for engulfment downstream of the FcγR.

Our results identify the involvement of a novel class I myosin, myosin IG in FcγR-mediated phagocytosis and suggest that this myosin is a downstream target of PI3K activity. Furthermore, both the motor domain and the PH-like domain of myosin IG contribute to the necessity of myosin IG for uptake following FcγR ligation.



Working Questions….

Is the localisation of and requirement for MyoIG at the phagocytic cup dependent on particle size?

What is the phosphoinositide binding specificity of the 'PH-like' domain?

Are these myosins involved in CR3-mediated phagocytosis?

References

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