Myosins

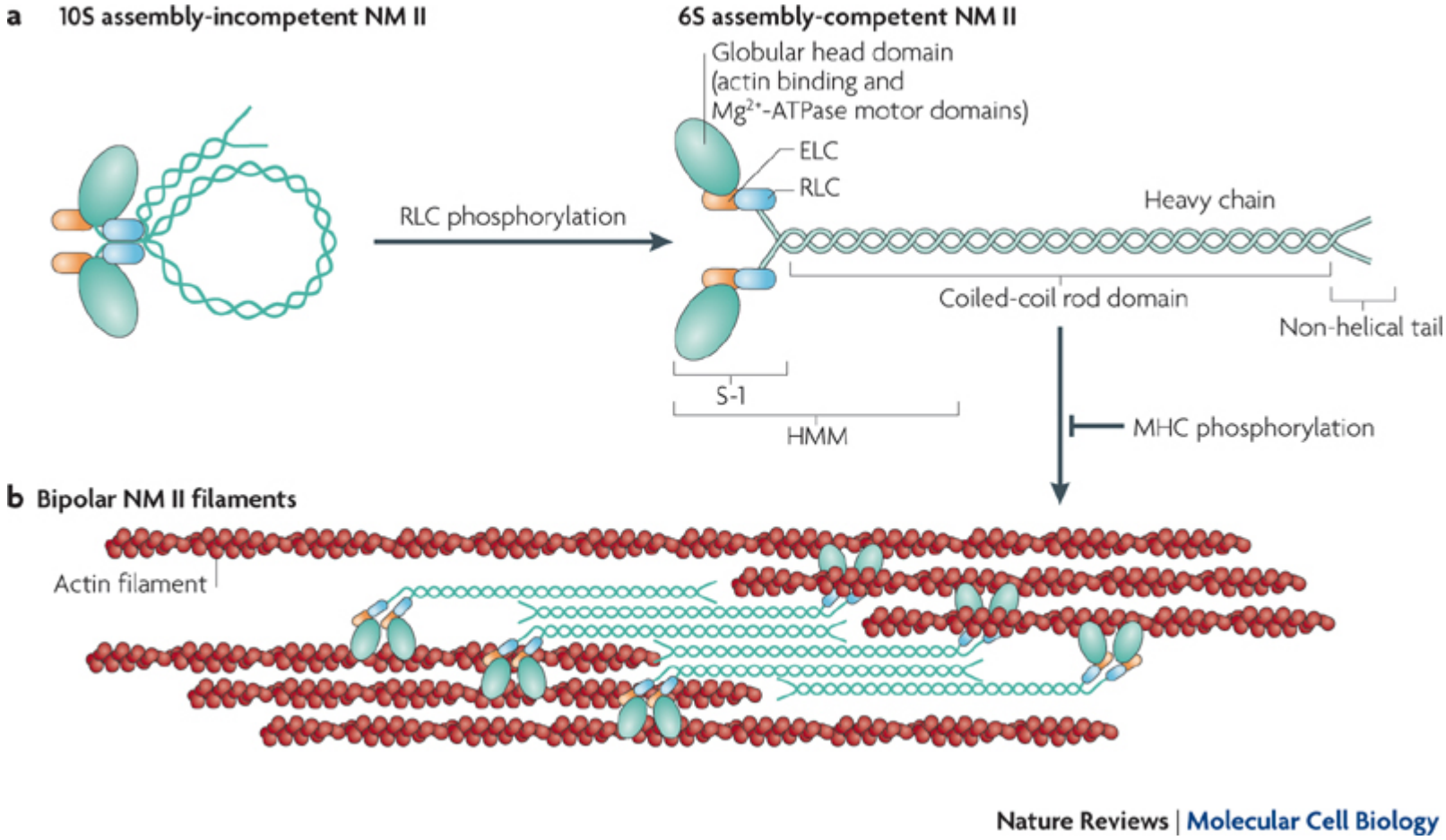
Myosins are actin-based motors with known or predicted roles in many types of eukaryotic motility. Along with actin polymerisation, myosins are thought to drive cellular movements

Myosins are motor proteins that interact with actin filaments and couple hydrolysis of ATP to conformational changes that result in the movement of myosin and an actin filament relative to each other.

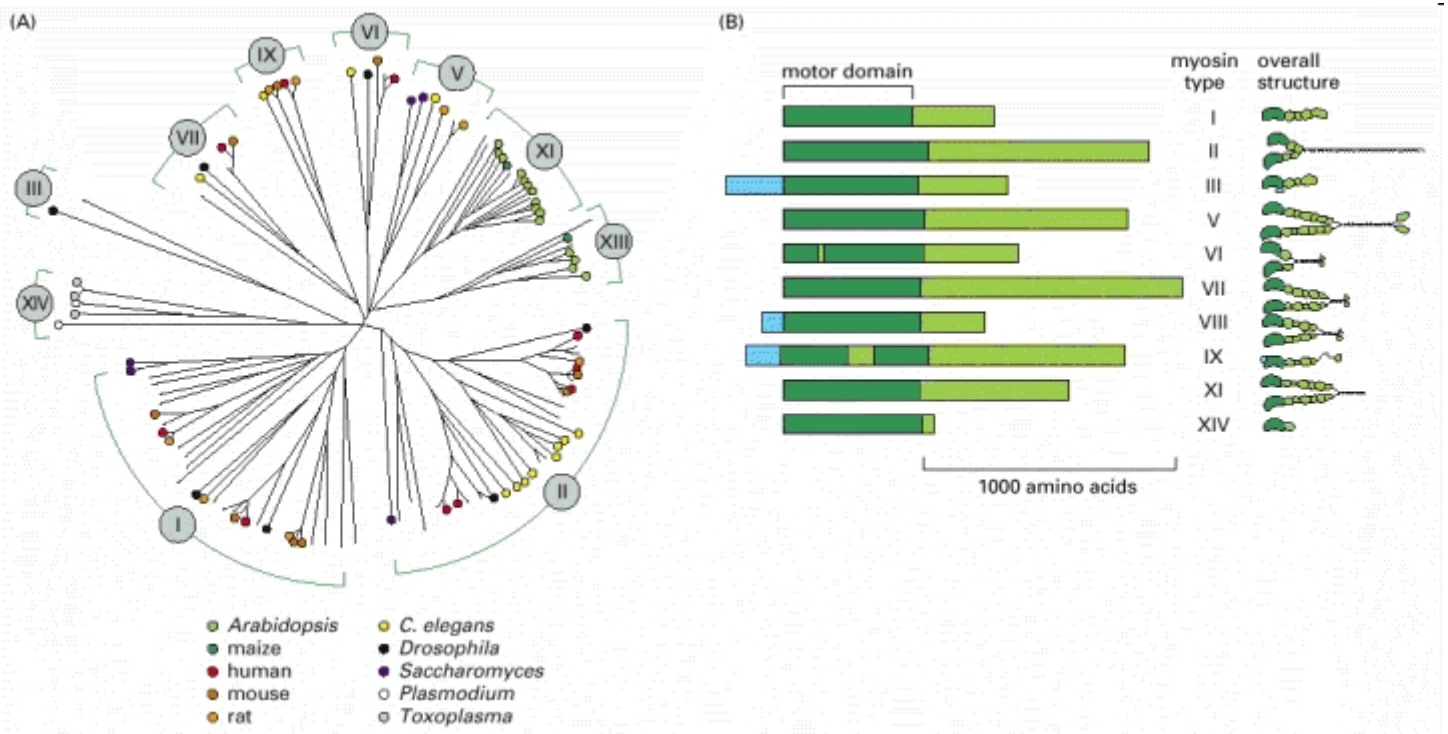
Known functions include cell adhesion, cell migration, cell division (cytokinesis), growth cone extension, maintenance of cell shape and phagocytosis

Also involved in signal transduction pathways, such as myosin II is required for F-actin polymerisation during CR3-mediated phagocytosis

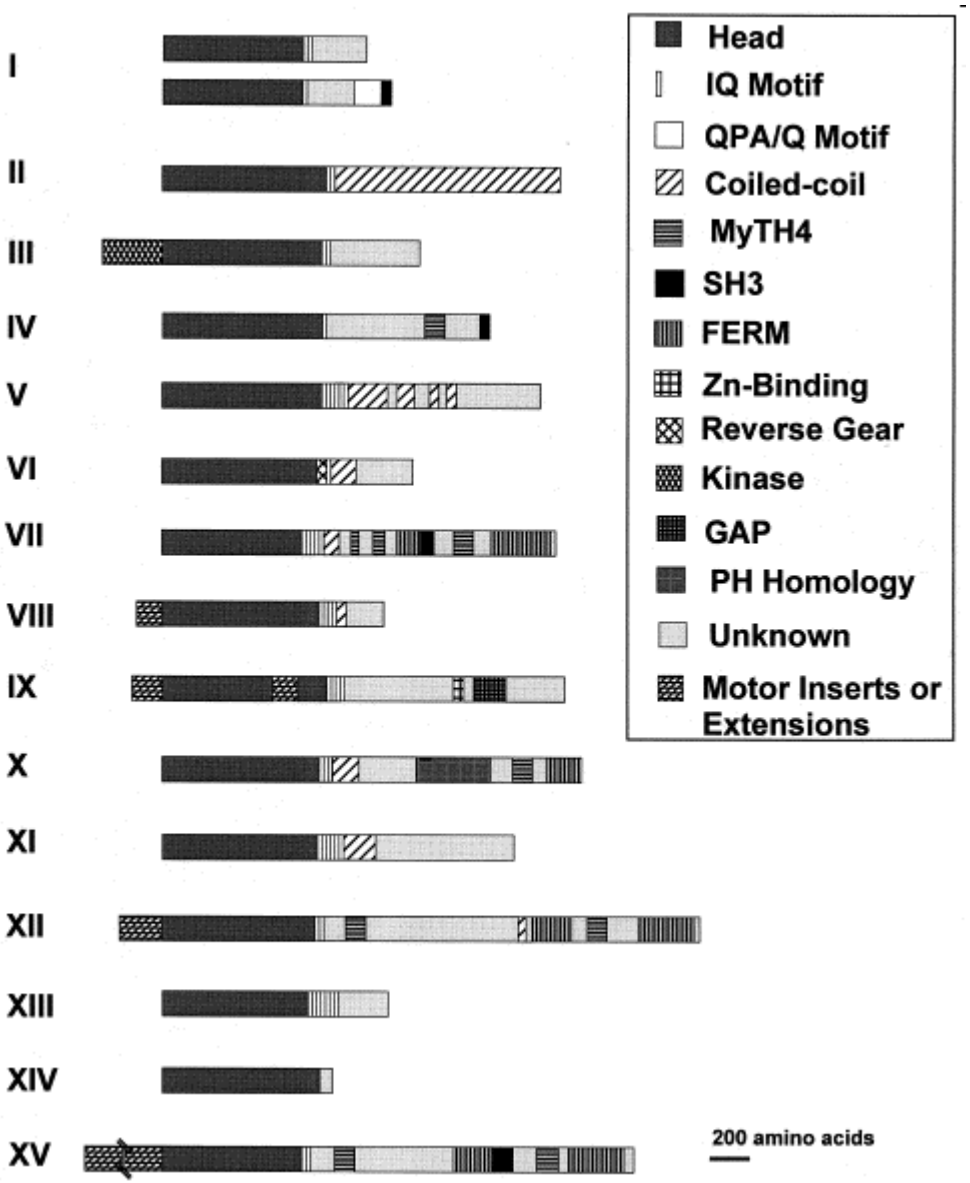
Domain structure of prototypical myosin II, a conventional myosin



Actin cross-linking and contractile functions



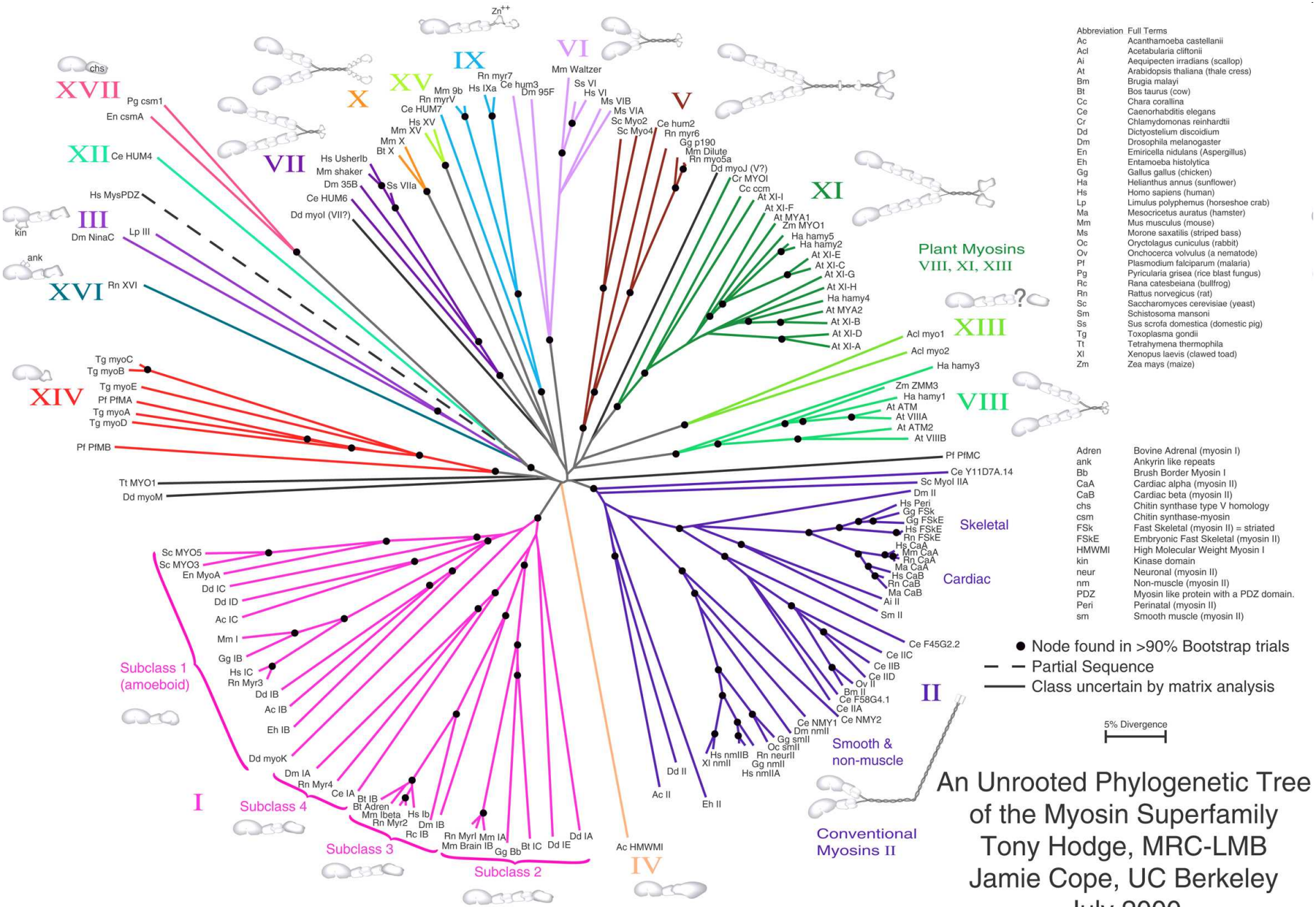
All myosins share similar motor domains (shown in *dark green),* but their C-terminal tails *(light green)* and N-terminal extensions *(light blue)* are very diverse. Many myosins form dimers, with two motor domains per molecule, but a few (such as I, IX, and XIV) seem to function as monomers, with just one motor domain.


I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
Head
IQ Motif
QPA/Q Motif
Coiled-coil
MyTH4
SH3
FERM
Zn-Binding
Reverse Gear
Kinase
GAP
PH Homology
Unknown
Motor Inserts or Extensions
200 amino acids

1) a motor region (head) whose core sequence is highly conserved in all the myosin classes. A core motor (catalytic) domain which interacts with actin and binds ATP.

2) a neck region (or 'lever arm') composed of a long helix of variable length depending on the number of IQ motifs (from none to six) which have the consensus sequence (IQxxxRGxxxR) and bind either light chains or calmodulin.

3) a tail region which is extremely variable in sequence length, domain composition and organisation. Although the identity and role(s) of many of the tail domains have yet to be established, they are believed to be involved in determining the cellular localisation (targeting) and function of the myosin (e.g. filament assembly, cargo binding). Myosins with tail regions containing predicted a-helical coiled coil domains are believed to be dimeric with two motor domains whereas those without a coiled coil region are monomeric with a single motor domain.



Abbreviation	Full Terms
Ac	Acanthamoeba castellanii
Acl	Acetabularia cliftonii
Ai	Aequipecten irradians (scallop)
At	Arabidopsis thaliana (thale cress)
Bm	Brugia malayi
Bt	Bos taurus (cow)
Cc	Chara corallina
Ce	Caenorhabditis elegans
Cr	Chlamydomonas reinhardtii
Dd	Dictyostelium discoidium
Dm	Drosophila melanogaster
En	Emiricella nidulans (Aspergillus)
Eh	Entamoeba histolytica
Gg	Gallus gallus (chicken)
Ha	Helianthus annus (sunflower)
Hs	Homo sapiens (human)
Lp	Limulus polyphemus (horseshoe crab)
Ma	Mesocricetus auratus (hamster)
Mm	Mus musculus (mouse)
Ms	Morone saxatilis (striped bass)
Oc	Oryctolagus cuniculus (rabbit)
Ov	Onchocerca volvulus (a nematode)
Pf	Plasmodium falciparum (malaria)
Pg	Pyricularia grisea (rice blast fungus)
Rc	Rana catesbeiana (bullfrog)
Rn	Rattus norvegicus (rat)
Sc	Saccharomyces cerevisiae (yeast)
Sm	Schistosoma mansoni
Ss	Sus scrofa domestica (domestic pig)
Tg	Toxoplasma gondii
Tt	Tetrahymena thermophila
Xl	Xenopus laevis (clawed toad)
Zm	Zea mays (maize)

Adren	Bovine Adrenal (myosin I)
ank	Ankyrin like repeats
Bb	Brush Border Myosin I
CaA	Cardiac alpha (myosin II)
CaB	Cardiac beta (myosin II)
chs	Chitin synthase type V homology
csm	Chitin synthase-myosin
FSk	Fast Skeletal (myosin II) = striated
FSkE	Embryonic Fast Skeletal (myosin II)
HMWMI	High Molecular Weight Myosin I
kin	Kinase domain
neur	Neuronal (myosin II)
nm	Non-muscle (myosin II)
PDZ	Myosin like protein with a PDZ domain.
Peri	Perinatal (myosin II)
sm	Smooth muscle (myosin II)

● Node found in >90% Bootstrap trials

– – Partial Sequence

——— Class uncertain by matrix analysis

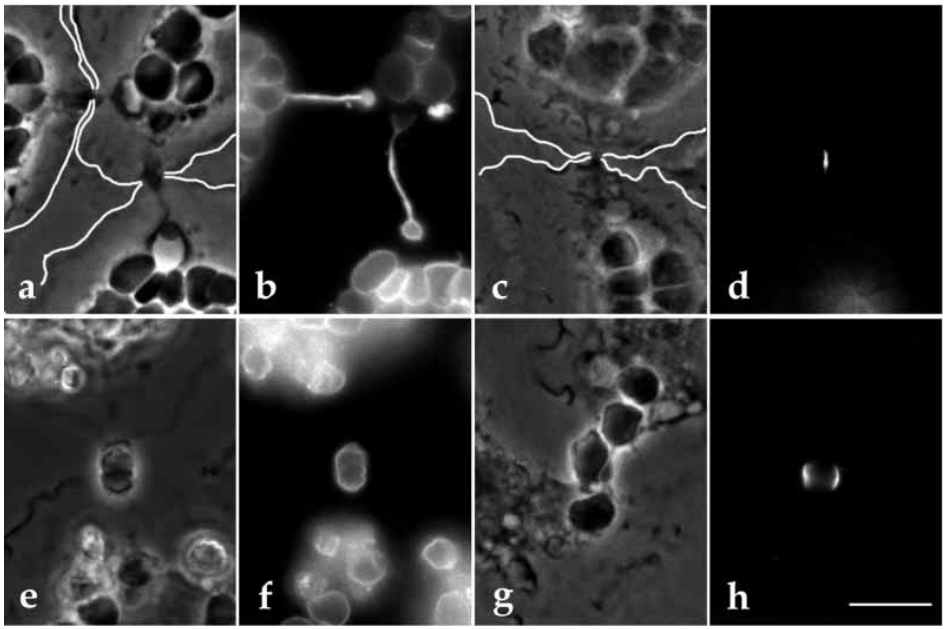
5% Divergence

An Unrooted Phylogenetic Tree of the Myosin Superfamily

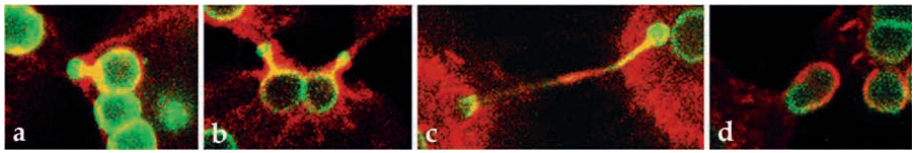
Tony Hodge, MRC-LMB
Jamie Cope, UC Berkeley

July 2000

Myosin-based contractility in phagocytosis

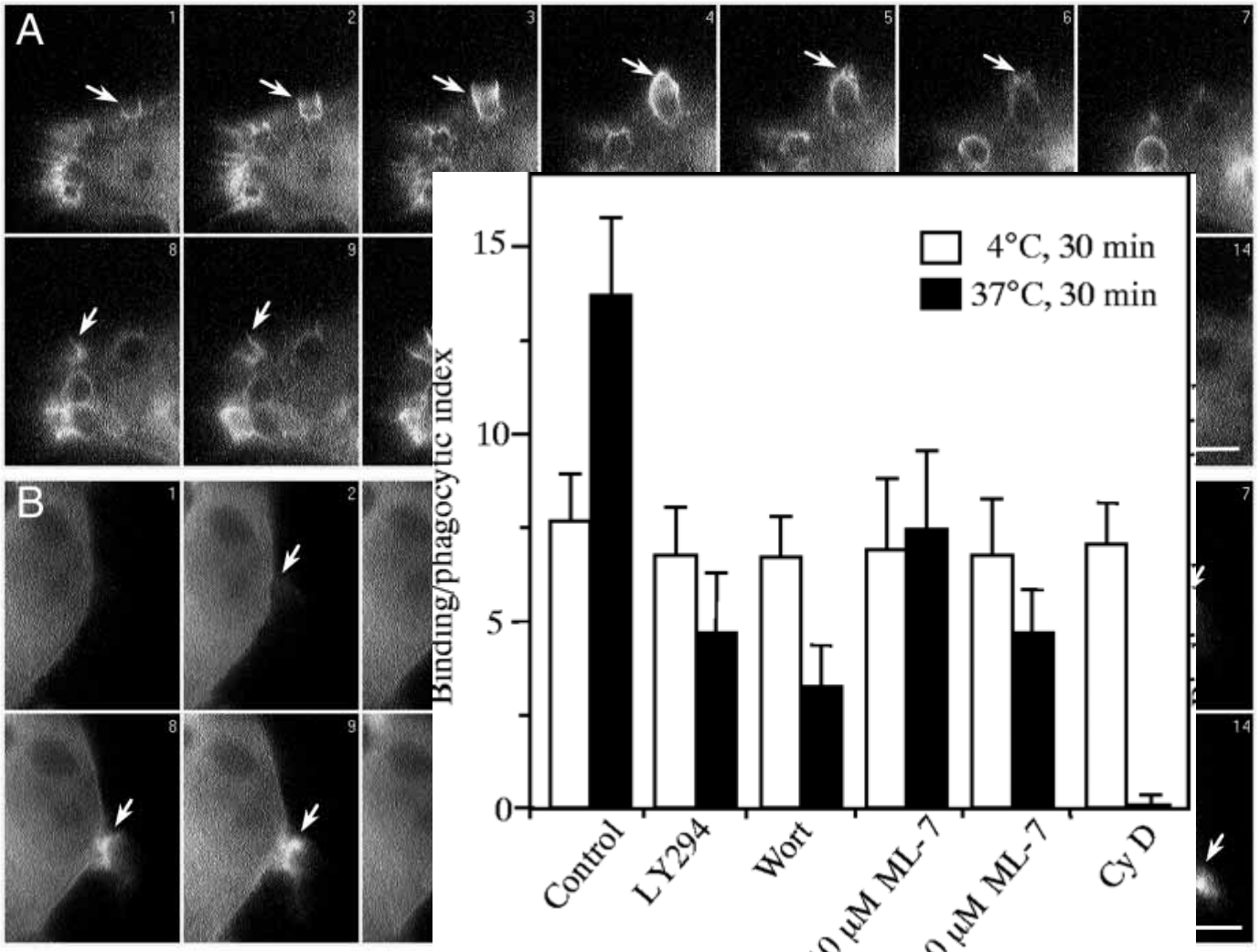


PI3K inhibitor, wortmannin blocks phagocytic cup constriction



Swanson et al., 1999

Myosin-based contractility in phagocytosis



Control

ML-7-treated

Time-lapse sequences of live macrophages expressing EGFP-actin, showing actin dynamics during FcγR-mediated phagocytosis of IgG-red blood cells.

Araki et al., 2003

Basis of myosin-based contractility in phagocytosis

In the presence of ML-7, slowed down and shortened pseudopod extension around the particles is observed but without apposition along the surface of the particles.

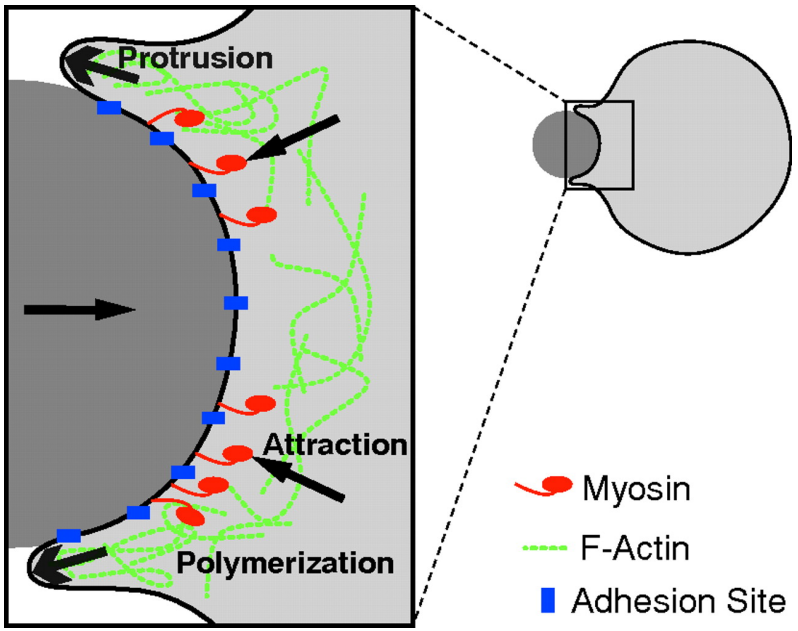
This finding
implies that sequential IgG-FcγR binding might not occur by itself, but requires forced zipper closure.

Two possible explanations are:

1. myosin-II contractile activity may promote the binding between the FcγR and ligands, to facilitate the efficient extension and subsequent closure of phagocytic cups.

2. the tight-fitting squeezing of the phagocytic cups pushes extra-particle fluid out of the phagosomes. This mechanism would decrease phagosomal volume and consequently increase intraphagosomal concentrations of superoxide, protons and hydrolases for bacterial killing and degradation.

Myosin-based contractility in phagocytosis

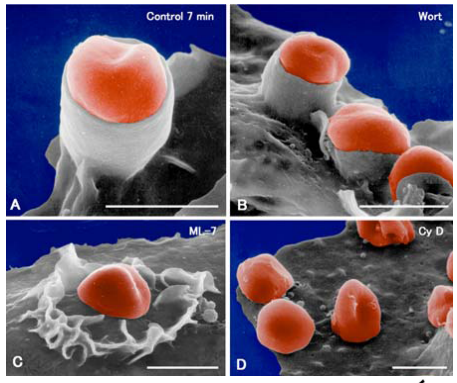


Taken from Herant et al., 2006 Journal of Cell Science

Mechanical model in neutrophils:

1) Cell-bead interface is stabilised by adhesion sites that act as anchors to the internal cytoskeleton,
2) 2) myosins draw down F-actin, pulling the bead into the cell or the cell onto the bead,
3) 3) polymerisation of new actin near the leading edge drives protrusion around the bead.

The myosin superfamily in humans



Required for uptake in neutrophils (“shaker-1” mice) but not detected in mouse macrophages

Needed for maximal pseudopod extension – mechanism unclear

Localised at phagocytic cups –role?

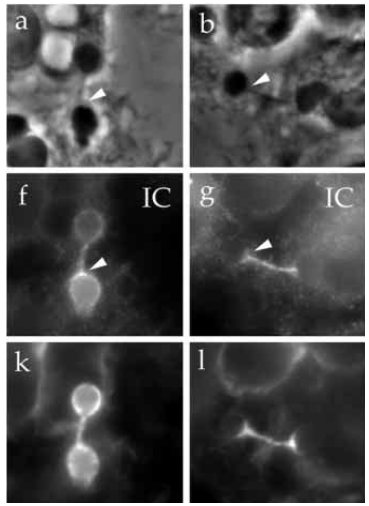
Contractile activity causing the squeezing motion

Mediates phagosomal movement

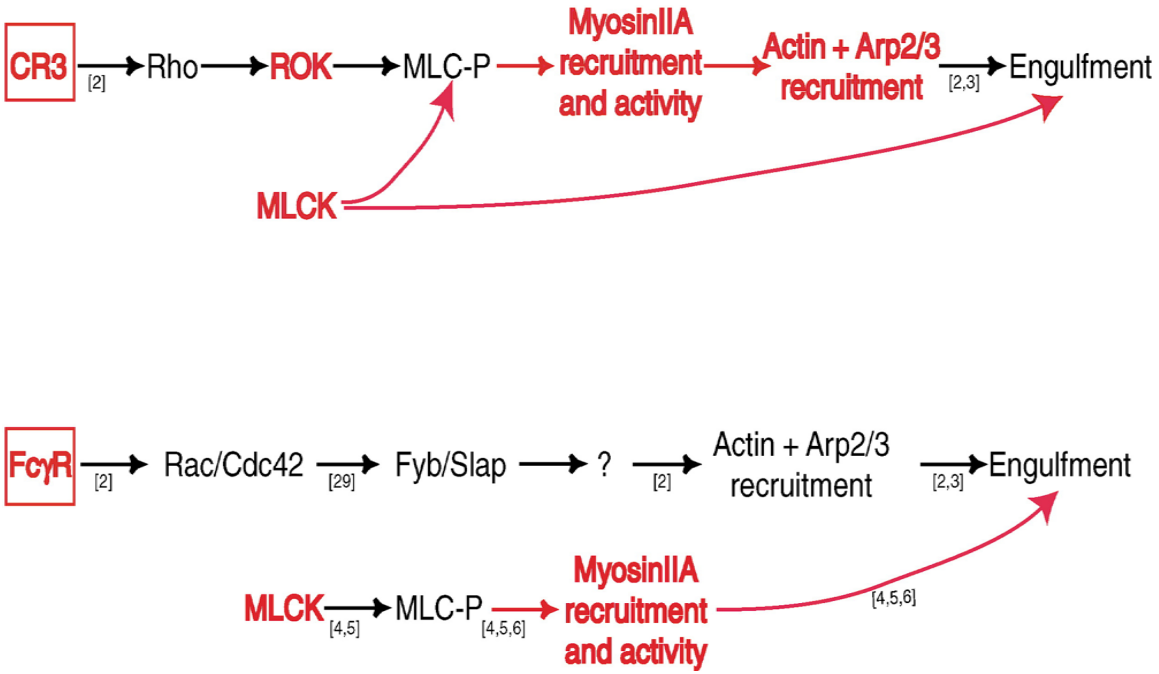
?

Decreased uptake in dendritic cells, not detected in BBDM

Involved in the ‘purse-string-like’ contraction



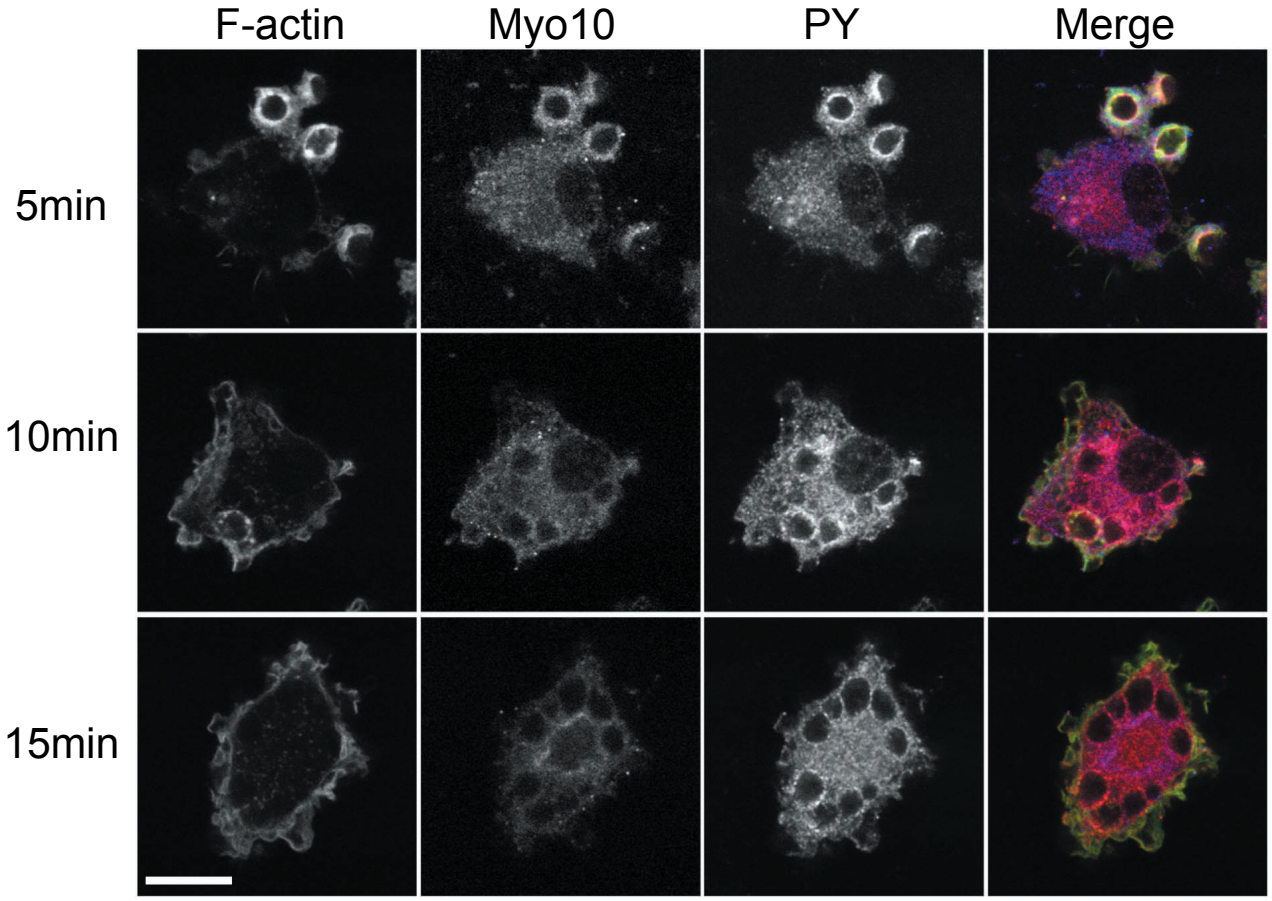
Myosin II in phagocytosis



Myosin II plays a role in particle internalisation during both FcγR- and CR3-mediated phagocytosis but is only required for actin cup assembly downstream of the CR3.

Olazabal et al., 2002

Myosin X in FcγR-mediated phagocytosis

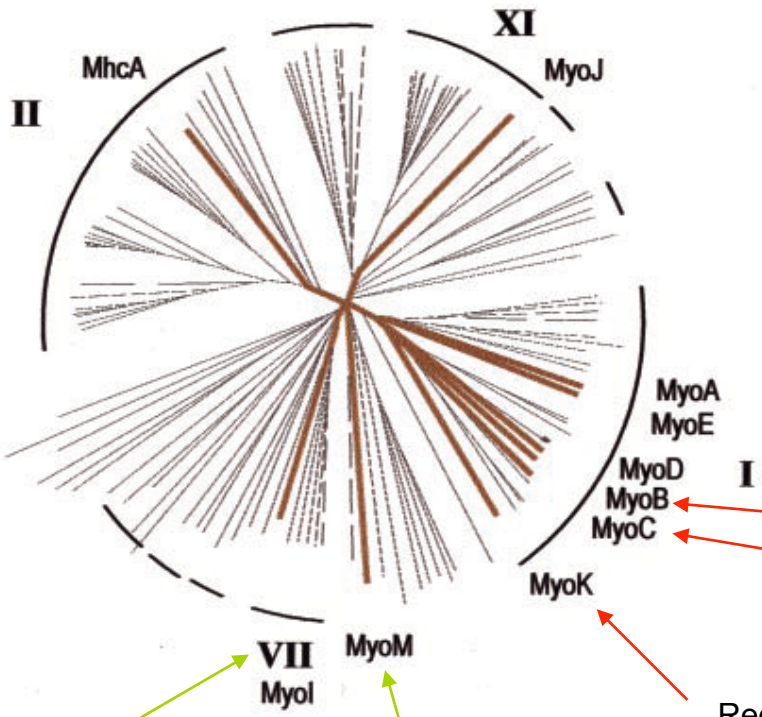


Myosin X is recruited to phagocytic cups and expression of a truncated myosin X tail inhibits the phagocytosis of large particles (6μm) but not small ones (2μm).

Predicted Mechanism: Myosin X binds to PIP3 in membrane through its PH domain the motor head domain engages actin filaments and moves towards the barbed ends

Cox et al., 2002

Myosins from *Dictyostelium*



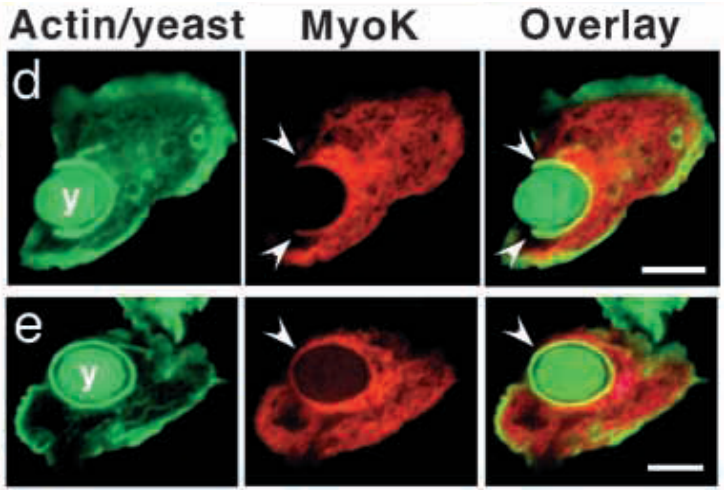
Localised in actin-rich cortical regions with non-redundant roles in phagocytosis

Shown to be important for uptake by *Dictyostelium* but not detected in mouse macrophages

Has a putative RhoGEF domain

Regulates the morphology of cortical actin

MyoK in Dictyostelium phagocytosis



myoK– cells as well as MyoK + cells phagocytosed about 30% less yeast cells than the wild-type. After 30 minutes, all three cell types reached a similar steady-state level of ingested yeasts, indicating that the phagocytic defect is in the rate of initial uptake and not in the downstream processing of phagosomes.

Schwarz et al., 2000

Aims:

Investigate cup closure

- Role of motor proteins in cup shape and closure using GFP-tagged myosins and confocal or SE microscopy.
- COS-7 cells transfected with FcγR during phagocytic challenge with IgG-opsonised beads.
- J774 macrophages transfected with siRNA to knockdown myosins
- J774 macrophages treated with myosin II inhibitors – blebbistatin, ML-7 or BDM.
- COS-7 cells transfected with WT FcγR or Fcγ(Y/F)2 or treated with cytochalasin D