Regulation and function of the fission yeast myosins

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Summary

It is now quarter of a century since the actin cytoskeleton was first described in the fission yeast, *Schizosaccharomyces pombe*. Since then, a substantial body of research has been undertaken on this tractable model organism, extending our knowledge of the organisation and function of the actomyosin cytoskeleton in fission yeast and eukaryotes in general. Yeast represents one of the simplest eukaryotic model systems that has been characterised to date, and its genome encodes genes for homologues of the majority of actin regulators and actin-binding proteins found in metazoan cells. The ease with which diverse methodologies can be used, together with the small number of myosins, makes fission yeast an attractive model system for actomyosin research and provides the opportunity to fully understand the biochemical and functional characteristics of all myosins within a single cell type. In this Commentary, we examine the differences between the five *S. pombe* myosins, and focus on how these reflect the diversity of their functions. We go on to examine the role that the actin cytoskeleton plays in regulating the myosin motor activity and function, and finally explore how research in this simple unicellular organism is providing insights into the substantial impacts these motors can have on development and viability in multicellular higher-order eukaryotes.

Key words: Tropomyosin, Fission yeast, *Schizosaccharomyces pombe*, Myosin, Actin

Actin' in the central role

As in all eukaryote cells, the actin cytoskeleton plays essential roles during the growth and life cycles of the fission yeast, *Schizosaccharomyces pombe*. This is clearly illustrated by the identification of key actin regulators in an early screen for cell division cycle mutants (Nurse et al., 1976). *S. pombe* has a single actin gene, *act1*$^{+}$ (Mertins and Gallwitz, 1987), which is ~90% identical to both the mammalian skeletal muscle actin and budding yeast actin. *S. pombe* actin is able to form filaments that are identical to actin that has been purified from muscle cells, but it is a weaker activator of the ATPase activity of the motor protein necessary for muscle contraction (Takaine and Mabuchi, 2007). Actin is capable of polymerising into long filaments, which can bundle together to form cables. The *S. pombe* actin cytoskeleton comprises three distinct actin structures: cortical patches, actin cables and a contractile cytokinetic actomyosin ring (CAR) (Marks and Hyams, 1985). This actin cytoskeleton is highly dynamic and reflects the pattern of growth and cytokinesis (Arai and Mabuchi, 2002; Marks and Hyams, 1985).

The cortical actin patches localise to the growing tips of the rod-shaped *S. pombe* cells and are sites of actin polymerisation (Pelham and Chang, 2001) and, together with actin-binding proteins (ABPs), are required for endocytosis (Kobori et al., 1989). After the fission yeast cell divides, growth occurs exclusively at the old cell end. Once the cell reaches a critical size, and has passed early G2 phase, growth begins at the new end, an event termed new end take off (NETO) (Mitchison and Nurse, 1985). Actin patch localisation reflects this growth pattern and, immediately after cell division, actin patches are only observed at the old end of the cell (Marks et al., 1986), whereas later on in the cell cycle they concentrate to both cell tips. Studies using a vital marker for cortical actin have shown that actin patches move through the cytoplasm on actin filaments (Pelham and Chang, 2001). During interphase, actin cables run along the entire length of the cell and are often associated with actin patches (Arai et al., 1998); most actin cables are orientated with their barbed ends towards the cell tips (Kamasaki et al., 2005).

At the onset of mitosis, the CAR begins to form. There are two proposed mechanisms for CAR formation. In the 'leading cable' mechanism, an aster-like structure branches out from the longitudinal actin cables at the cell equator (Arai and Mabuchi, 2002; Skoumpla et al., 2007). As the aster extends to form the primary ring, accumulated actin filaments are bundled together (Pelham and Chang, 2002) to form a ring comprising two semicircular populations of parallel filaments, but with opposite directionality (Kamasaki et al., 2007). Longitudinal actin cables that are oriented with their barbed ends towards the cell equator are also seen attached to the growing ring (Kamasaki et al., 2005). Recent research has led to the formulation of an alternative hypothesis called the 'search and capture' mechanism, in which actin filaments extend from nodes composed of a number of proteins, including the anillin-like protein Mid1, myosin II and the formin Cdc12. Growing actin filaments are captured by myosin II in adjacent nodes, which pulls the nodes towards each other and allows the bundling of the growing actin filaments into cables. This process is repeated, resulting in the coalescing of nodes and actin cables into the CAR (Coffman et al., 2009; Huang et al., 2008; Vashishtha and Fischetti, 1993; Vavylonis et al., 2008; Wu et al., 2006).

The debate is still ongoing with regard to the precise mechanism by which the actin ring is assembled, and it is not inconceivable that a combination of these two proposed mechanisms is at work within the cell. Indeed, recent studies have shown both pathways might co-exist, with the notion that the 'search and capture' pathway is dependent upon the anillin-like protein Mid1, and that the 'leading cable' mechanism requires the activity of the septation initiation network (Hachet and Simanis, 2008; Huang et al., 2008). It is appealing to follow the actin polymer dynamics in live fission

yeast cells to obtain a deeper understanding of their precise regulation and dynamic behaviour. However, it should be born in mind that all of the fluorescently labelled *S. pombe* actin markers described to date have a stabilising effect on actin polymers in the cell (Coulton et al., 2010).

Actin polymers and their functions are regulated by a variety of ABPs. These include the Arp2/3 complex, profilin, formins, actin-depolymerising factor (ADF; also known as cofilin), actin capping and bundling proteins, and tropomyosin. The effects of these ABPs on actin dynamics are beyond the scope of this article, but for further details see previous comprehensive overviews (dos Remedios et al., 2003; Pollard et al., 2000). Here, we discuss a specialised group of actin-associated motor proteins, the myosins, and the regulation of their interactions with the actin polymer.

Get your motors running…

Every eukaryote possessing an actin-based cytoskeleton has at least one myosin. These are evolutionarily conserved motor proteins that engage in a plethora of tissue-specific cellular functions. The diversity of the cellular functions in which they engage is reflected in the variety of biophysical behaviours and structures among the different myosins, which are categorised into different myosin classes according to their overall sequence. Aligning the sequences of their C-terminal tail domains represents a simple method for classification of the myosins (Table 1; Box 1), as this domain imparts the ability of a myosin to engage in non-motor functions (e.g. to dimerise or to bind to substrates, such as cargoes, membranes or chaperones).

It is thus perhaps surprising that the same classification is obtained when the different myosin N-terminal actin-binding ATPase motor domains are aligned. Divergence of the motor domain sequences is likely to cause differences in the motor activity of a myosin (e.g. speed and the ability to take multiple steps). For example, differences in the motor domain allow some myosin classes to move processively, as either a dimer or monomer (e.g. myosin V, VI or IX), to work in concert with other myosins to exert continual force (e.g. myosin II), or to act as a tension sensor within the cell (e.g. myosin I) (Coluccio and Geeves, 1999). For further details, see the previous detailed reviews on the impact of diversity between different myosin sequences (De La Cruz and Ostap, 2004; Krendel and Mooseker, 2005). However, as more genome sequences become available, the total number of myosin classes is ever expanding, and the tally of 35 classes (Odronitz and Kollmar, 2007) is likely to be exceeded soon with the increase in specific myosins that are discovered in novel organisms.

Fission yeast contains five myosins from the three classes I, II and V (Box 1; Table 1). This myosin set is likely to represent the minimal ‘fleet’ of motors that is required for the execution of actomyosin functions within a eukaryote cell. Their small number has made the study of myosins in yeast very attractive, as there is the potential to correlate the biochemical properties of all actin motors with their cellular functions within a single cell type. Since the discovery of the first *S. pombe* myosin more than a decade ago (Kitayama et al., 1997; May et al., 1997), the fission yeast sequencing project has identified the five myosin encoding genes, with each motor named according to its classification (i.e. class I, Myo1; class II, Myo2 and Myp2; and class V, Myo51 and Myo52).

A class of its own: Myo1

The discovery of a novel myosin, which diverged substantially from the conventional class II myosins, in *Acanthamoeba*, unlocked a Pandora’s box of new classes of myosins for the burgeoning motor research community to explore (Pollard and Korn, 1973). Representatives of the class I myosins (i.e. the first of the current 34 classes of ‘unconventional’ myosins) have subsequently been identified in all animals and fungi studied to date (Odronitz and Kollmar, 2007). The gene encoding *S. pombe* Myo1 was identified from data acquired during the fission yeast genome sequencing project (Lee et al., 2000; Toya et al., 2001). Typical class I myosins are monomeric proteins, which comprise either one or two calmodulin-binding IQ domains, and a tail region containing a number of functional domains that facilitate its functions (e.g. membrane binding). In a manner similar to the budding yeast class I myosins (Myo3 and Myo5), Myo1 has a long tail region ending in an acidic C-terminal tip, which plays an important role in stimulating Arp2/3-complex-dependent actin polymerisation. Myo1 shares this task with Wsp1, the fission yeast homologue of the Wiskott–Aldrich syndrome protein (WASp), with Myo1 and Wsp1 acting in parallel pathways to regulate the activation of Arp2/3 and the distribution of cortical actin patches at the sites of new cell growth (Fig. 1) (Lee et al., 2000; Sirotkin et al., 2005). This Myo1 function is reflected in the phenotype of *myo1Δ* cells, which lack a polarised distribution of cortical actin patches as well as a polarised pattern of cell growth. Similarly, Myo1 associates with actin patches (Lee et al., 2000; Sirotkin et al., 2005; Toya et al., 2001), where it not only promotes actin polymerisation but also plays a key role in membrane remodelling and endocytosis (Attanapola et al., 2009; Codlin et al., 2008). Its association with the membrane is highly dynamic; each interaction lasts ~14 seconds. However, no substantial lateral Myo1 movements have been observed, indicating that it does not travel along actin cables during

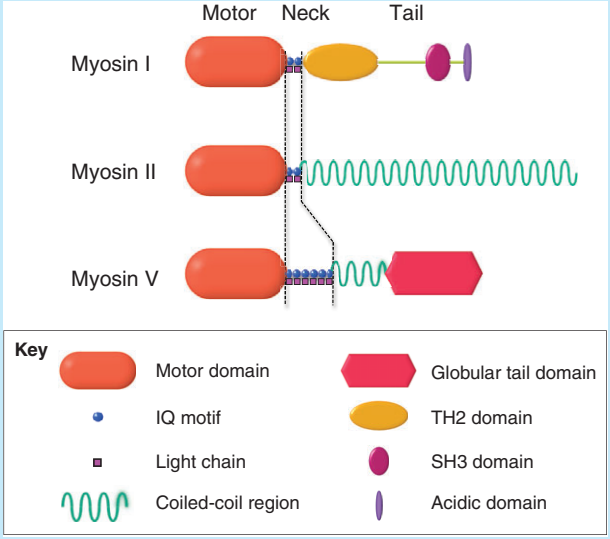
Table 1. Summary of localisation, regulation and functions of the *S. pombe* myosins

Cass	Myosin	Light chains	Regulation by phosphorylation	Regulation by Cdc8	Cellular function
I	Myo1	Cam1	Ser361 (TEDS site)	Does not associate with Cdc8–actin polymers	Arp2/3 activation; endocytosis
II	Myo2	Cdc4 and Rlc1	Myo2, Ser1444; Rlc1, Ser35 and Ser36	Acetylated Cdc8 inhibits the ATPase activity of both class II myosins	Provides contractile force during cytokinesis
II	Myp2	Cdc4 and Rlc1	Unknown		Maintaining fidelity of CAR during cytokinesis
V	Myo51	CAR and meiotic SPBs	Unknown	Both myosin V motors move along Cdc8–actin polymers; Cdc8 plays no role in modulating the motor activity of myosin V	Regulating spore formation
V	Myo52	Motile foci concentrating at sites of cell growth	Unknown		

Box 1. Yeast myosin domains

Motor domain

The motor domain is the powerhouse of the myosin protein. This region confers the ability to associate with actin in an ATPase-dependent manner. ATP hydrolysis results in a conformational change within the head domain that is translated into a movement (amplified by the myosin neck region), which allows the protein to exert force against actin. The motor activity can vary substantially between different classes of myosin, which allows the myosins to undertake a diverse range of functions within the cell.



Neck region

The myosin neck region typically consists of a variable number of IQ domains, to which calmodulin or calmodulin-like light chains associate in a Ca^{2+}-dependent manner. This association of a light chain with the IQ motifs within the neck region not only increases the stiffness of this 'lever arm' but also prevents the tail region from folding back on itself, which would inhibit motor activity. Calmodulin is the light chain for most non-conventional myosins (yeast class I and V myosins), whereas class II myosins associate with two specific factors, an essential light chain and a regulatory light chain. When bound to the IQ domains, the light chains increase the rigidity of the neck region, which allows amplification of the power stroke generated from the motor domain.

Tail domain

The tail domains diverge substantially between different myosin classes. The tail domain imparts the ability to engage in non-motor functions and thus determines the specific protein–protein interactions undertaken (e.g. dimerisation, binding to cargoes and association with membranes).

this period (Attanapola et al., 2009; Sirotkin et al., 2005; Takeda and Chang, 2005). As in all class I myosins, the tail of Myo1 contains a conserved tail homology domain (TH1 domain), which is required for the association of Myo1 with dynamic lipid regions. The TH1 domain thus permits Myo1-dependent deformation of cellular membranes during endocytosis and, probably, specialised membrane reorganisation during meiosis (Codlin et al., 2008; Itadani et al., 2006; Lee et al., 2000; Takeda and Chang, 2005; Toya et al., 2001).

The mechanisms by which Myo1 activity and function are regulated are only just starting to be elucidated. As with other long-tail class I myosins, Myo1 possesses two IQ domains adjacent to the C-terminal end of its motor domain. Biochemical analysis of myosin I from metazoan organisms has demonstrated that calmodulin associates with these IQ domains in a Ca^{2+}-dependent manner. This results in an increase in the structural rigidity of the IQ domain region, which allows it to act as a lever arm to amplify the magnitude of the motor power stroke. In addition, this Ca^{2+} sensitivity can affect the ATPase cycle of mammalian myosin I, altering the affinity of this myosin to modulate its function within the cell (Adamek et al., 2008). However, although the fission yeast calmodulin Cam1 associates with Myo1, and is required for its localisation (Toya et al., 2001), the biophysical consequences of this interaction have yet to be explored.

The activities of all myosins are regulated by interactions with specific light chains. These calmodulin-like proteins (and in some cases calmodulin itself) associate with IQ motifs within the neck region, where they not only increase the stiffness of the 'lever arm' but also prevent the tail region from folding back on itself to inhibit motor activity (Sellers and Knight, 2007; Thirumurugan et al., 2006). In addition to light chain binding, biochemical and phosphoproteome analyses have uncovered multiple phosphorylation sites in Myo1 (S. L. Attanapola, PhD thesis, University of Kent, 2009) (Wilson-Grady et al., 2008). To date, the consequences of phosphorylation on Myo1 activity have only been explored for a conserved TEDS site motif within its motor domain. This conserved TEDS site is located on an α-helical loop at the actin–myosin interface, which is only found in myosins from simple unicellular organisms (Bement and Mooseker, 1995). For *S. pombe* Myo1, phosphorylation of this site (Ser361) is dependent on the STE20 kinases Nak1 and Pak1 (also known as Orb3 and Orb2, respectively), and modulates the affinity of Myo1 for actin, thereby regulating its endocytic function (Attanapola et al., 2009). Mutations within the TEDS site can bypass the inhibitory effect that tropomyosin has on the ability of Myo1 to bind to longer actin cables; mutation of the conserved TEDS-site serine residue to an aspartic acid residue (to mimic phosphorylation) results in Myo1 decorating actin cables throughout the cell (Attanapola et al., 2009; Clayton et al., 2010). The relative contribution of different ABPs to myosin regulation will be explored in more detail below. Myo1 motor activity is not necessary for its role in promoting actin polymerisation, but the mechanism by which it contributes to other cellular processes (e.g. endocytosis) is not yet known.

Myo2 and Myp2: the dynamic duo of the ring cycle

Class II myosins provide the contractile force in muscle tissue. They were the first myosins to be identified and therefore have been the most extensively studied. These myosins possess long α-helical coiled-coil tails, allowing them to form homodimers, which are then incorporated into heavy chain filaments within muscle cells. In non-muscle metazoan cells, myosin II maintains cortical rigidity, as well as providing the contractile force during cytokinesis. Unlike other unicellular organisms, which only possess a single myosin II, *S. pombe* has two class II myosins, Myo2 and Myp2. Myo2 was the first fission yeast myosin to be identified and is its only essential myosin. However, as in budding yeast, the requirement for myosin II can be bypassed, as demonstrated by the fact that mutants lacking the entire *myo2*$^{+}$ gene acquire mutations within other genes, which in turn allow growth and colony formation (Kitayama et al., 1997; May et al., 1997; Tolliday

et al., 2003). The second myosin II, Myp2 (also known as Myo3) (Bezanilla et al., 1997; Motegi et al., 1997), plays a more subtle role during cell division, as *myp2Δ* cells are capable of dividing normally but are susceptible to stress and challenges to CAR integrity (Bezanilla and Pollard, 2000; Fujita et al., 2002; Martin-Garcia and Valdivieso, 2006; Mulvihill and Hyams, 2003; Mulvihill et al., 2000). Myo2 and Myp2 are integral components of the cytokinetic machinery at the CAR (Bezanilla et al., 2000; Motegi et al., 2000); although all myosins localise to the division site during cell division, only the class II myosins play crucial roles in facilitating the contraction of the CAR (Lord et al., 2005). Myp2 is thought to exist as a monomer, whereas the tail of Myo2 is capable of forming stable dimers in vitro (Bezanilla and Pollard, 2000). These Myo2 tail dimers can be localised to, and incorporated into, the CAR independently of actin (Mulvihill et al., 2001a; Naqvi et al., 1999), but it is currently unclear whether they are also incorporated into longer myosin II filaments at this structure.

Myo2 and Myp2 localise to the cell equator independently and at different times during mitosis (Bezanilla et al., 1997; Kitayama et al., 1997; Motegi et al., 2000; Wu et al., 2003). Myo2 first appears at the division site as dots that colocalise with the fission yeast anillin-like protein Mid1 (Wong et al., 2002). At the onset of anaphase, Myo2 then potentially interacts with formin-associated actin seeds and tropomyosin to complete formation of the CAR (Mulvihill and Hyams, 2002; Skau et al., 2009; Stark et al., 2010). Once the CAR is formed, Myo2 can be rapidly exchanged (Wong et al., 2002), a process that is controlled by the conserved UCS-domain-containing protein Rng3, which is required for ensuring CAR integrity (Lord and Pollard, 2004; Lord et al., 2008; Wong et al., 2000). Myp2 is recruited to the CAR later in mitosis (Bezanilla et al., 2000; Motegi et al., 2000; Wu et al., 2003), where it plays a role in the later stages of cytokinesis.

In a manner similar to that of Myo1, the activity of *S. pombe* myosin II is regulated by phosphorylation and association with distinct light chains. As with the muscle myosins, Myo2 and Myp2 each associate with an essential (Cdc4) light chain and a regulatory (Rlc1) light chain. However, unlike the case in muscles, the mechanisms by which these proteins regulate Myo2 localisation and its activity are not yet fully understood. (Burgess et al., 2007; D'Souza et al., 2001; Le Goff et al., 2000; Liu et al., 2006; McCollum et al., 1995; Naqvi et al., 1999; Naqvi et al., 2000; Thirumurugan et al., 2006). Interestingly, and different from the process in higher-order eukaryotes, in which phosphorylation of myosin II heavy and light chains affect the cellular function of the motor, phosphorylation of Cdc4 has no impact upon the cell (McCollum et al., 1999; Motegi et al., 2004; Sladewski et al., 2009). Rlc1 phosphorylation increases Myo2 motility in vitro, and phosphorylation of the myosin tail domain regulates the timing of Myo2 incorporation into the CAR and its subsequent constriction (Motegi et al., 2004; Sladewski et al., 2009). The identity of the protein kinase(s) that are responsible for the phosphorylation events that trigger CAR formation and constriction remain unknown, but it is worth noting that the fission yeast polo-like kinase Plo1 and the septation initiation network have been implicated in regulating the phosphorylation of the Myo2 regulator Mid1 (Bahler et al., 1998; Motegi et al., 2004).

Are we there yet? Myosin V delivers the goods

The remaining fission yeast myosins belong to the class V myosins, which dimerise to 'walk' along actin cables and deliver cargoes to discrete cellular locations. Adjacent to their motor domains, they have a long neck region that is composed of up to six calmodulin-binding IQ motifs, which precede a coiled-coil domain (permitting dimer formation) and a C-terminal globular tail domain (GTD) (Box 1), which binds, either directly or through adapter proteins, to its molecular cargo. In higher-order metazoan cells, class V myosins typically have a high duty ratio (i.e. they associate with actin for a large part of their ATPase cycle) and are capable of moving processively on actin (i.e. take multiple steps before disassociating from the actin polymer). However, there is evidence suggesting that class V myosins from yeast and simpler eukaryotes represent a subset of class V myosins that is only capable of moving processively in the presence of cargo proteins (Hodges et al., 2009; Reck-Peterson et al., 2001; Taft et al., 2008; Toth et al., 2005).

The two fission yeast class V myosins, Myo51 (also known as Myo5) and Myo52 (also known as Myo4), were first described, approximately a decade ago, as motor proteins that have distinct localisation patterns within the cell. Myo51 is a component of the CAR and does not show any observable movement within vegetative cells, whereas Myo52 moves around the cell and accumulates at regions of cell growth and cell wall deposition (Fig. 2) (Motegi et al., 2001; Win et al., 2001). Deletion of the gene encoding Myo51 has no discernable effect on cell growth, whereas cells lacking Myo52 are temperature sensitive and have

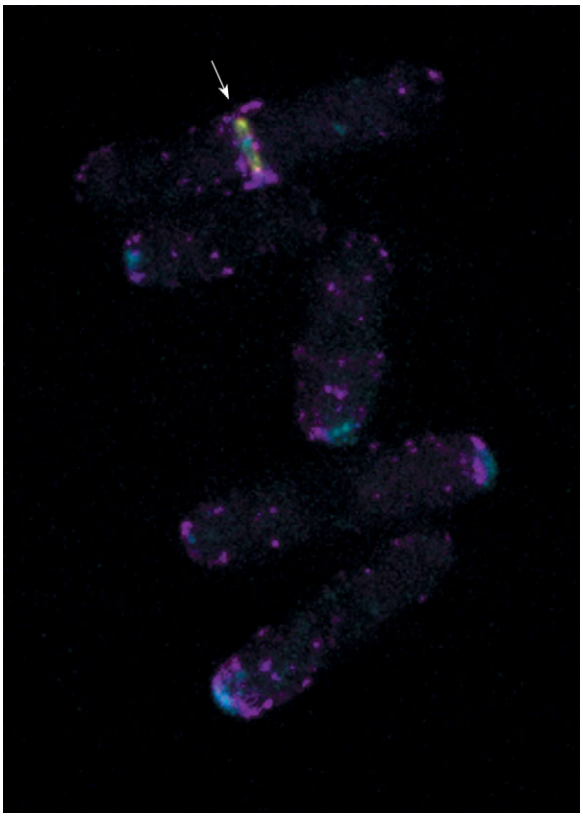


Fig. 1. *S. pombe* myosins. Localisation of myosin I (Myo1, cyan), myosin II (Myo2, yellow) and myosin V (Myo52, magenta) in live yeast cells in which the chromosomal copy of each myosin gene has been fused to cDNA encoding different fluorescent proteins. Myo1 is present at sites of endocytosis and actin patch polymerisation. Myo2 is only observed at the CAR (arrow) during mitosis, where it is involved in the maintenance and constriction of the ring. Myo52 is found in the cytoplasm and associated with actin filaments; it is also located at sites of cells growth, to which it delivers various cargoes.

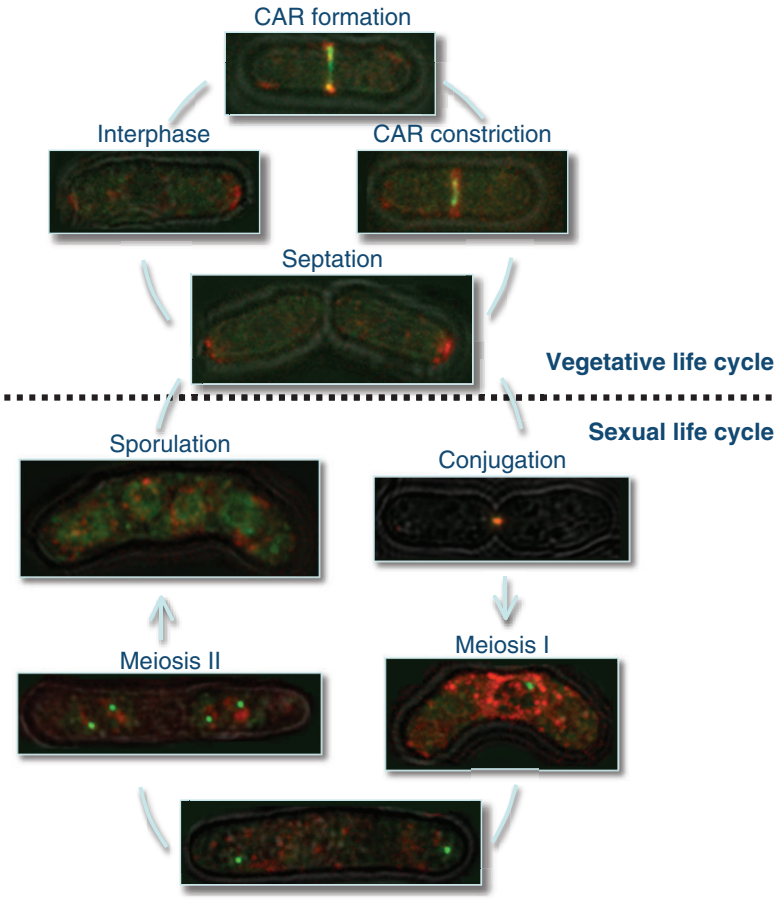


Fig. 2. Class V myosin localisation during vegetative and sexual life cycles. Myo51 and Myo52 have discrete localisation patterns during vegetative and meiotic life cycles, but colocalise at the site of cell fusion. Shown here are yeast cells in which Myo52 (red) exclusively accumulates in regions of cell growth that are enriched in cortical actin, such as the ends of growing cells, the site of septum formation during cell division, the site of cell fusion during mating and around the nucleus during meiosis. This contrasts dramatically with the localisation of Myo51 (green), which is only seen during the vegetative life cycle when it is associated with the CAR during mitosis. Myo51 colocalises with Myo52 at the site of conjugation and is localised to the SPBs during meiotic nuclear division, where it has a role in coordinating spore formation. Myo51 is the only fungal myosin that has been reported to associate with SPBs.

morphological defects. Indeed, in its original description, Myo52 was suggested to have a role in directing the localisation of the α-glucan synthase Mok1 to promote appropriate cell wall synthesis (Win et al., 2001). Myo52 has subsequently been implicated in a variety of different cellular processes. As well as its role in cytokinesis, ensuring the appropriate delivery of the β-1,3-glucan synthase Bgs1 (also known as Cps1) to the site of cell division (Mulvihill et al., 2006), it has been implicated in vacuole organisation and maintenance (Motegi et al., 2001; Mulvihill et al., 2001b), and in transport of the SNARE protein synaptobrevin to its appropriate cellular location (Edamatsu and Toyoshima, 2003). In addition, a new function of *S. pombe* Myo52 in regulating microtubule dynamics has recently been described (Martin-Garcia and Mulvihill, 2009). Through interaction with the ubiquitin receptor protein Dph1, Myo52 regulates the turnover of the *S. pombe* CLIP-170 homologue Tip1, a key regulator of microtubule function, to promote the dynamic instability of microtubules at the cell tips. This observation indicates that there is crosstalk between microtubules and the actin cytoskeleton to ensure the correct regulation and maintenance of cell growth and polarisation.

In contrast to Myo52, the function of Myo51 during the mitotic cell cycle has proven to be more elusive. However, changes in global gene expression provided an initial clue to its function during the sexual life cycle (Mata et al., 2002). As for Myo52, Myo51 was shown to localise to the tip of mating cells, where both myosins play a role in promoting cell fusion (Doyle et al., 2009). After completion of prophase I, Myo51 is localised to segregating meiotic spindle pole bodies (SPBs), where it plays a role in coordinating the temporal and spatial organisation of spore formation (Fig. 2). The maintenance of Myo51 localisation at the SPBs is not dependent on actin but on the microtubule cytoskeleton (Doyle et al., 2009), indicating that Myo51 itself is a cargo for microtubule-associated motor proteins (e.g. kinesins or dynein).

Analysis of the movements of thousands of Myo52 foci has determined that the mean cellular velocity for Myo52 is ~0.55 μm per second (Grallert et al., 2007; Motegi et al., 2001). Intriguingly, it has recently been reported that Myo52 can also move with higher velocity (~2 μm per second), raising the possibility that different Myo52 populations travel at different speeds, depending upon their cargo or local cellular environment (Clayton et al., 2010). This is consistent with the observed myosin-V-dependent movement of vesicles in budding yeast (Schott et al., 2002), which move exclusively at the higher velocity.

As is the case for the class I and II myosins, the activity of each class V myosin is regulated by light chains associating with the IQ motifs within the myosin neck domain. As for Myo1, calmodulin acts as a light chain for both Myo51 and Myo52, and associates with each of the IQ motifs within the neck region of these class V myosins (Grallert et al., 2007). In addition, the myosin II essential light chain (Cdc4) has also been shown to interact with the IQ region of Myo51, and this might reflect the exclusive localisation of Myo51 to the CAR during the vegetative growth cycle (Box 1; Table 1) (D'Souza et al., 2001). Surprisingly, however, removal of all IQ motifs from Myo52 has no discernable effect on its cellular velocity, which indicates that calmodulin is dispensable for Myo52 motility and that its neck region, which in other class V myosins acts as a lever arm, is not required for its movement within the cell (Grallert et al., 2007). The role of phosphorylation in the regulation

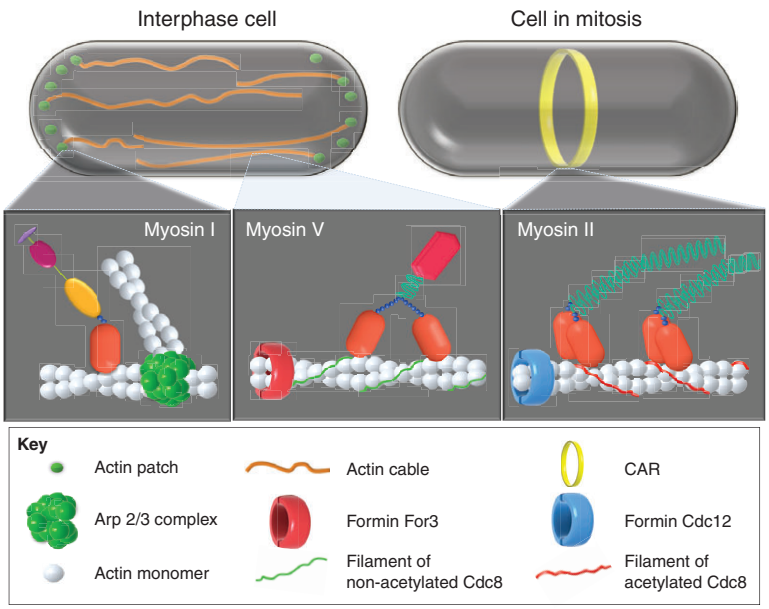


Fig. 3. Schematic illustration of yeast myosin localisation to discrete actin structures. The fission yeast class I myosin Myo1 exclusively associates with cortical actin patches (green circles in the interphase cell). These patches comprise a network of branched actin filaments, which are nucleated by, and branch from, the Arp2/3 complex, but lack Cdc8. The class V myosins Myo51 and Myo52 travel throughout the interphase cytoplasm along actin cables or filaments that are nucleated by the formin For3. Filaments of dimerised non-acetylated Cdc8 coil around and stabilise these actin structures, but do not affect myosin V mobility. Both of the *S. pombe* class II myosins normally only associate with actin cables within the CAR that are nucleated by the mitotic formin Cdc12. Polymers of acetylated stabilised Cdc8 polymers not only maintain the integrity of the CAR, but also sit in the closed position on the actin polymer and thereby regulate the motor activity of the class II myosins. The representation of myosin subdomains is as in Box 1.

of fission yeast class V myosins is currently unknown; however, the composition of the track on which they move has been shown to affect their cellular mobility (Clayton et al., 2010; Coulton et al., 2010).

The laws of the road: tropomyosin regulates actin–myosin interactions

It is now emerging that actin polymers can play an important role in regulating the motor activity of myosins within non-muscle cells, including *S. pombe*. The different actin structures within fission yeast are formed by distinct actin-polymerising proteins. Cortical actin patches are nucleated by the Arp2/3 complex, and interphase actin filaments by the formin For3, whereas the CAR is composed of filaments that are seeded by the mitosis-specific formin Cdc12, which exclusively localises to the cell equator during cell division. These different actin polymers are associated with different *S. pombe* myosins; Myo1 is associated with cortical patches, Myo52 with interphase cables, and both Myo2 and Myp2 interact with the CAR. This suggests that differences in the architecture of these distinct actin–ABP complexes provide the basis for the molecular mechanisms underlying the functions of the different myosins (Fig. 3). Further evidence for this is provided by the fact that conditional mutants in the gene encoding the fission yeast tropomyosin Cdc8 affect the localisation and motility of myosins II and V (Motegi et al., 2000; Pelham and Chang, 2001). In addition, in strains that lack either For3 or functional Cdc12, the localisation of Myo52 or Myo2, respectively, is also disrupted (Motegi et al., 2000; Pelham and Chang, 2001).

The yeast tropomyosin Cdc8 is an ABP that regulates actin dynamics and the interactions between actin and myosin. Fission yeast has a single tropomyosin gene, which was first identified (Balasubramanian et al., 1992) in a complementation assay using human fibroblast tropomyosin. Yeast cells that do not have functional Cdc8 lack actin cables and continue to grow in length, but they are characterised by a swollen ‘dumbbell’ shape and are also unable to undergo cytokinesis. Cdc8 localises to cytoplasmic actin cables and to the CAR. Cdc8 is also found in patches within the cytoplasm, but these patches do not colocalise with cortical actin patches (Arai and Mabuchi, 2002; Skoumpla et al., 2007). Cdc8 is required for stabilising actin cables and maintaining polarised cell growth (Chang et al., 1996; Pelham and Chang, 2001). As in budding yeast, *S. pombe* actin cables that are stabilised by Cdc8 serve as ‘tracks’ for myosin motor proteins (Motegi et al., 2001; Pelham and Chang, 2001).

Cdc8 polymerises around the actin filaments that form longitudinal cables during interphase and the actin filaments around the CAR during cell division (Skoumpla et al., 2007), but is absent from cortical patches. Recent research has shown that the presence of the actin crosslinker fimbrin excludes Cdc8 from these actin structures (Skau and Kovar, 2010) by causing localised deformations in the actin polymer, which is inconsistent with a cooperative binding model of Cdc8 to actin (Pruyne, 2008).

Cdc8 expression levels do not vary throughout the cell cycle, and, at any given point, ~80% of Cdc8 is acetylated, whereas ~20% is unacetylated (Skoumpla et al., 2007). Acetylation greatly increases the affinity of Cdc8 for actin and stabilises a particular configuration of Cdc8 polymers on actin, which is able to regulate myosin II activity (Skoumpla et al., 2007; Stark et al., 2010). In this so-called closed position, acetylated Cdc8 polymers bind to actin in a way that prevents myosin from binding strongly to actin. Biochemical assays have revealed that, in contrast to skeletal muscle or budding yeast tropomyosins, unacetylated Cdc8 is also capable of associating with actin, albeit weakly (Skoumpla et al., 2007). Subsequently, it has been shown that the acetylated and unacetylated forms of Cdc8 interact with actin at discrete locations within the *S. pombe* cell (Coulton et al., 2010). Acetylated Cdc8 is observed predominantly at the CAR, whereas the unacetylated form is seen exclusively on interphase actin filaments. It has also been shown that Cdc8 acetylation is required to regulate the in vivo motility of class II myosins but has no effect on the motility of class I and V myosins. In vitro studies have also revealed that fimbrin competes with tropomyosin for actin binding, which might be the underlying reason for the lack of Cdc8 association with actin patches in vivo (Clayton et al., 2010; Skau and Kovar, 2010). Therefore, together with other ABPs, the fission yeast Cdc8 acts as a key regulator of actin–myosin interactions.

Motoring on into the future

Biochemical, biophysical and cell biological studies are providing intriguing insights into the structure–function relationship of the fission yeast myosins and the effects actin polymer composition has on their activity. Each of the fission yeast myosins undertakes clearly distinct roles within the cell and, moreover, each class of myosin localises to different types of actin structures within the cell. This specificity is in part determined by the myosin motor protein itself, but is also influenced by regulatory actin-binding factors, such as tropomyosin. Comparisons with studies from metazoan cells are now beginning to reveal conservations in myosin function and regulation, and not merely in sequence. Indeed, the observation that different tropomyosin isoforms interact with distinct actin structures in different metazoan cell types (Bach et al., 2009; Creed et al., 2008) is reminiscent of the observation in fission yeast that the acetylated and unacetylated forms of Cdc8 associate with distinct classes of actin polymers.

Recent research is just beginning to uncover new cellular functions for all fission yeast myosins and the roles of the respective motor activities. For instance, novel functions for myosins have been described, including regulating the dynamic properties of the microtubule cytoskeleton. It is now becoming apparent that the different classes of myosins are able to modulate the dynamics of both the actin and microtubule cytoskeletons (Lantz and Miller, 1998; Wu et al., 2005), thereby affecting cell polarity and growth, and also pointing towards a conservation in function from yeast to humans.

It is probable that the opportunities allowed by studying actomyosin function in fission yeast will help to uncover other mechanisms of regulation and reveal new functions for components of the cytoskeleton. The power of yeast genetics, and the ability to rapidly identify new myosin V mutants, continues to be useful in furthering our understanding of how these motor proteins work within the cell. In addition, yeast also has the potential to lead the way in system-wide research, for example, by using combined modern proteomic, molecular genetic and microscopy techniques to define the entire set of cargoes for the two class V myosins.

Thus, the journey exploring actomyosin regulation and function is far from over. Taking advantage of the powerful tools allowed by the simple unicellular yeast is likely to pay a key part in unlocking the hidden secrets of these conserved molecular machines.

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