The evolution of compositionally and functionally distinct actin filaments

Peter W. Gunning¹*, Umesh Ghoshdastider²*, Shane Whitaker¹, David Popp² and Robert C. Robinson²,³,‡

ABSTRACT
The actin filament is astonishingly well conserved across a diverse set of eukaryotic species. It has essentially remained unchanged in the billion years that separate yeast, Arabidopsis and man. In contrast, bacterial actin-like proteins have diverged to the extreme, and many of them are not readily identified from sequence-based homology searches. Here, we present phylogenetic analyses that point to an evolutionary drive to diversify actin filament composition across kingdoms. Bacteria use a one-filament-one-function system to create distinct filament systems within a single cell. In contrast, eukaryotic actin is a universal force provider in a wide range of processes. In plants, there has been an expansion of the number of closely related actin genes, whereas in fungi and metazoa diversification in tropomyosins has increased the compositional variety in actin filament systems. Both mechanisms dictate the subset of actin-binding proteins that interact with each filament type, leading to specialization in function. In this Hypothesis, we thus propose that different mechanisms were selected in bacteria, plants and metazoa, which achieved actin filament compositional variation leading to the expansion of their functional diversity.

KEY WORDS: Actin, Evolution, Filament, Tropomyosin

Introduction
The functions of biological filaments derive from their abilities to form linear polymers. These structures provide strength, architecture and location as scaffolding components within cells, which participate in ordering and shaping compartments and organelles, as well as localizing molecules to their appropriate physical locations. A second property of some polymers is that directed polymerization, and in some instances depolymerization, can provide pushing and pulling forces that can be integrated into biological processes in which movement is a necessary component (Pollard and Cooper, 2009). Essentially, if an elongating filament is pointed at an object, then a force will be exerted on that object from the growing filament. If enough filaments are arranged with their growing ends directed at that object, then the object will move, provided that the filament system is relatively immobile in comparison to the object – the filament system needs traction in order to push. Classic examples of the integration of the forces created from polymerization into biological systems include: (1) actin filaments polymerizing at the leading edge of a moving eukaryotic cell, driving the membrane forwards (Pollard and Cooper, 2009); and (2) during E. coli cell division, the bacterial actin-like filament ParM capturing an R1 plasmid at both ends, propelling the two copies apart to ensure faithful DNA inheritance (Salje et al., 2010).

The ability to form a protein polymer is a trivial feat in biology. The protein simply needs to possess a head-to-tail binding site for itself that allows for many copies of the protein to assemble into a geometry that is longer than it is wide. Several self-associating metabolic enzymes are known in bacteria, and it is from such origins that actins and tubulins are speculated to have evolved (Barry and Gitai, 2011). However in general, linear self-association interactions will have been selected against during evolution because polymerization is non-productive in most areas of protein function. Amyloid fibrils and sickle-cell hemoglobin are archetypal examples of the detrimental results of proteins self-associating without a biological justification.

To be a useful biological polymer, the protein needs to be dynamic in its self-association and dissociation so that it can be assembled and disassembled in an appropriate spatial and temporal manner. Tubulin and actin use mechanisms whereby they hydrolyze their bound nucleotides, which regulate the stability of the filaments by altering the conformation of their protomers through allosteric mechanisms (Mitchison and Kirschner, 1984; Pardee and Spudich, 1982). On polymerization, the ATPase activity of actin is activated, which creates a timing record of the progress of polymerization (Pardee and Spudich, 1982). The newly formed portion of the filament will still be mostly bound to ATP, the slightly older portion bound to ADP and phosphate, and the oldest portion is bound to ADP following phosphate release (Bugyi and Carlier, 2010). A similar mechanism exists for GTP bound to tubulin and its hydrolysis to GDP (Desai and Mitchison, 1997). The relative stabilities of these different nucleotide-bound forms within the filaments dictate the assembly and disassembly rates and disassembly mechanisms of actins and tubulins. Intermediate filaments use different and largely unknown mechanisms, which in part involve phosphorylation and ubiquitylation (Omary et al., 2006; Windoffer et al., 2011). The nucleotide hydrolysis mechanisms allow for the disassembly of actin and tubulin to be regulated on a filament-by-filament basis as opposed to phosphorylation and ubiquitylation, which work more on a bulk population basis. Thus the crucial properties of the actin polymer (and the microtubule) are the dynamic abilities to linearly self-associate, to be able to time the dissociation within single filaments and to recycle the monomers through nucleotide exchange. Furthermore, actins and tubulins generally form polar filaments. This adds directionality to the force generation during polymerization and directionality to the interactions with other proteins, such as eukaryotic motor proteins.

The roles of actin-like and tubulin-like filaments have been interchangeable during evolution. The contractile ring in mammalian cells consists, among other proteins, of actin nucleators (formins), myosin and actin filaments (Pollard, 2010). In contrast, the Z-ring in many bacteria is largely formed from the tubulin homolog FtsZ (Lutkenhaus et al., 2012). Conversely,
tubulin is involved in chromosome segregation in mammals (Wittmann et al., 2001), whereas the actin-like protein ParM is involved in plasmid segregation in many bacteria (Salje et al., 2010). Thus, the biological functions of actins and tubulins have been somewhat transposable during evolution, which should not be surprising given that the filament systems share similar basic characteristics.

Here, we use phylogenetic and structural arguments to form a hypothesis with regard to the specialization of different actins during evolution. The bacterial ParMs will have diverged due to the relative lack of constraints within these simple plasmid-segregating systems. In multi-plasmid settings, there might even have been positive selective pressures to diversify in order to faithfully ensure inheritance of each distinct plasmid. In contrast, eukaryotic actin is a universal force provider that is integrated into many biological processes. We invoke structural arguments to postulate that once the force of actin polymerization had been harnessed for more than one biological function, the central player, actin, had little chance to evolve without compromising one or more of those functions. We expand this hypothesis to explore the possibility that during eukaryotic multicellularization, different routes may have been taken that resulted in the expansion of functionality of actin. We identify two potential mechanisms: (1) the expansion in the number of closely related actin genes in plants, and (2) the introduction of tropomyosin, which forms a co-polymer with actin, in fungi and metazoa. Both mechanisms have expanded the composition of the actin filament through regulating the subset of actin-binding proteins that are capable of interacting with each filament type.

**Bacterial actins**

Many bacteria contain three types of actin-like filaments, MreB, FtsA and ParM. MreB is involved in maintenance of cell shape and cell wall synthesis (Doi et al., 1988), FtsA aids FtsZ in forming the Z-ring (Addinall and Lutkenhaus, 1996), whereas ParM is the aforementioned polymerizing motor that segregates large DNA plasmids (Jensen and Gerdes, 1997). A fourth class of actin, MamK, forms the scaffolding component of the magnetosome in a few bacterial species (Komeili et al., 2006). Sequence analysis of these bacterial actins reveals that these types of bacterial actins cluster on different branches of the phylogenetic tree (Fig. 1A). ParMs are highly divergent, whereas FtsAs and MreBs have diverged to an intermediate extent with respect to ParMs and the highly conserved eukaryotic actins. This indicates that FtsA, MreB and ParM have diverged at different rates. The clustering of FtsAs and MreBs on separate branches of the phylogenetic tree suggests that the two proteins had already diverged in the common ancestor of these bacteria. However, ParMs (Fig. 1A) are less related than the whole genome sequences of their respective species (Fig. 1B), which is not surprising, because ParMs are plasmid-encoded proteins and thus might have been subjected to different selective pressures compared to genome-encoded proteins.

The reasons for these different rates of diversification might, at least in part, be attributable to function. One factor that will have affected the divergence of bacterial actin is the number of interacting partners. ParMs only need to interact with themselves and the filament end-binding proteins (ParR) (Salje et al., 2010). This leaves large exposed surfaces on the sides of ParM filaments that are not subject to selective pressures from interaction partners. Thus ParMs have experienced relatively few constraints and will have diverged, at least in part, through genetic drift (Kimura, 1968). In contrast, MreB is a bacterial cell-shape-determining protein that recruits the machinery responsible for synthesizing the cell wall protein peptidoglycan and interacts with FtsZ during Z-ring contraction (Fenton and Gerdes, 2013). Similarly, FtsA is a second FtsZ-interacting protein that helps assemble the cell division machinery and coordinates cell wall synthesis during the process (Lutkenhaus et al., 2012; Szwedziak et al., 2012). These activities involve protein–protein and protein–membrane associations with the sides of the filaments. Such interactions will have placed greater restrictions on the divergence of the surfaces of MreB and FtsA filaments.

In addition, we speculate that in a multi-plasmid setting, the ParM segregating machineries might have been additionally subjected to positive selection to diverge in order for each distinct plasmid to be faithfully inherited within a single bacterium. One potential example of this are the two *Bacillus vietnamensis* ParMs, which are especially highly divergent (Fig. 1). Essentially, when each plasmid encodes a unique polymerizing motor (ParM) that can be harnessed by a distinct DNA-binding protein (ParR) bound to an exclusive DNA sequence on that plasmid (*parC*), then segregation of each distinct plasmid will be reproducible, irrespective of whether a second type of plasmid exists in the same cell. These highly divergent ParM amino acid sequences (Fig. 1) have recently been shown to translate into equally divergent filament architectures (Gayathri et al., 2013; Popp et al., 2010a; Popp et al., 2012; Popp et al., 2010b), which adds some weight to the hypothesis that an element of positive selection to diverge might have existed between selected ParMs.

The emergence of distinct bacterial actins, which co-exist in the same cell, is compatible with the interpretation that a single actin-like filament did not meet the functional requirements of the evolving bacterial cell. This might, at its simplest, reflect a need to be able to spatially separate, and therefore independently regulate, the assembly and function of different bacterial actin filaments in a ‘one-filament-one-function’ manner. For instance, the timing and location of assembly of the plasmid-segregation filament ParM will be different to those required for cell wall synthesis and cell division, and thus for MreB and FtsA. The divergence of the bacterial actins has ensured that they will form independent homopolymers, as protomer interfaces and helical parameters are variable among the classes of actin-like filaments (Gayathri et al., 2013; Popp and Robinson, 2011; Szwedziak et al., 2012; van den Ent et al., 2001). Thus accomplishment of a specific biological function has provided the context in which the host actins (MreB and FtsA) have diverged and have become optimized for their specialized function. This might also be the case for the ParMs that are encoded on plasmids that contain an essential trait, such as antibiotic resistance, where the interests of the cell and plasmid have to be coordinated.

**Eukaryotic actin regulation**

Eukaryotes utilize actin as a universal scaffolding and force-providing molecule that is harnessed for a wide range of processes that require form and force. This approach has a distinct benefit in that a single pool of polymerizable actin can be maintained. In contrast, the bacterial ‘one-filament-one-function’ system requires a distinct pool of each actin-like protein to be sustained for each unique filament system and its associated biological process. Limitations in resources will restrict the number of actin-like protein pools a cell is able to simultaneously maintain, and in turn limit the number of processes in which filaments can participate. Thus, adoption of the eukaryotic ‘universal-actin-pool’ system allows force and scaffolding functions to be incorporated into a greater number of biological processes.

One requisite to adopting the universal-actin-pool system is that a more complex level of regulation is required, because actin needs to
Fig. 1. Maximum likelihood phylogenetic relationships between the actin-like bacterial proteins ParM, MreB, FtsA and eukaryotic actin. (A) ParMs have diverged far more than the species in which they are found (B). In contrast, eukaryotic actins have hardly diverged in relation to their species. Horizontal branch lengths indicate the degree of divergence amongst the different proteins, and numbers indicate the branch support. The average numbers of substitutions per residue (SR) are 0.81, 1.16 and 4.10 for the MreB, FtsA and ParM branches, respectively, and 0.08 between S. pombe (red hash) and human (blue hashes) cytoplasmic actins. Relatedness within FtsAs and MreBs, but often not for ParMs, generally follows the relatedness of the species (compare blue and red plus symbols, SRs for these proteins between two species are 0.10, 0.45 and 5.4, respectively), except where two MreBs are found in a single species (black asterisks, SR 1.05), which suggests lineage-specific gene duplication. There, a likely diversification in function will have led to greater sequence variation. ParMs from the same host species can be highly divergent (green asterisks, SR 4.55). The sequences were aligned in MUSCLE 3.8 (Edgar, 2004), phylogenetic trees were constructed using FastTree 2.1.7 (Price et al., 2010) and visualized by FigTree (http://tree.bio.ed.ac.uk/software/figtree/). Relationships between species were generated in SUPERFAMILY (Wilson et al., 2009). Accession codes for this figure, and subsequent figures, are given in supplementary material Table S1.
be maintained at higher levels than needed for many of the individual processes. This greater level of control will have resulted from the emergence of the actin-regulating proteins. Actin filament nucleation machineries, such as formins and Arp2/3 and its activators, allow the harnessing of the force of polymerization for specific processes. Steric obstruction of spontaneous non-productive polymerization from actin filaments and monomers are carried out by capping proteins and profilin, respectively, as failure to prevent unregulated polymerization would deplete the pool of polymerization-competent actin (Xue and Robinson, 2013), leading to intracellular chaos. The emergence of the actin-based motor myosin expanded the possibilities for force generation in biological systems.

For many of the actin-regulating factors, there are now crystal or electron microscopy reconstruction structures in their actin-bound state available. When their interacting-surfaces on actin are plotted against the actin sequence it becomes immediately apparent that almost every amino acid of actin is buried within a protomer or important for an actin–actin contact and/or for an actin–regulating-protein contact (Fig. 2). Actin surface residues for which no binding partner has been defined, might nevertheless have a role in the processive elongation of filaments by formins, as these proteins slide over the exterior of actin. Such interactions might not be revealed in the static crystal structures. Furthermore, many actin residues will be important for flexibility and function at other stages, such as during polymerization (Oda et al., 2009; Xue et al., 2014), ATP hydrolysis and transition from ATP- to ADP-bound filament structures (Pollard et al., 2000), as well as in the folding process of G-actin (Egelman, 2003). Human γ-actin and fission yeast actin share 91.2% overall identity. This subdivides into 93.8%, 91.2% and 87.0% identity for residues buried in a monomer, residues buried in the filament interface, and surface exposed residues, respectively (Fig. 2). This conservation of surface residues adds weight to the hypothesis that interactions between actin and its binding partners have dictated the evolution of actin in eukaryotes (Egelman, 2003; Galkin et al., 2002; Hightower and Meagher, 1986). Thus, a minimal set of actin-regulating proteins will have 'locked-in' the structure and sequence of actin as a highly connected hub during the evolution of eukaryotes (Carlson et al., 2006). In support of this hypothesis, deletions of Arp2/3 (Madania et al., 1999; Winter et al., 1999), cofilin (Lappalainen et al., 1997), formins (Evangelista et al., 2002), profilin (Magdolen et al., 1988), myosin

![Fig. 2. The majority of actin residues have a known function. The alignment shows the human isoforms of cytoplasmic actin (ACTB and ACTG1) in comparison to fission yeast actin (Pombe), with the interaction properties of each amino acid indicated below the alignment. The majority of residues are involved in known actin–actin or actin–binding-partner contacts. Buried (B, blue) indicates buried residues in the G-actin structure (Wang et al., 2010), F-actin (F, mustard) indicates residues that are in the F-actin interfaces (von der Ecken et al., 2014), Arp2/3 (A, pink) (Robinson et al., 2001; Volkmann et al., 2001), Formins (F, green) (Otomo et al., 2005; Thompson et al., 2013), Myosin (M, orange) (Behrmann et al., 2012), Cofilin (T, cyan) (Paavilainen et al., 2008) and Profilin (P, red) (Schutt et al., 1993) indicate interacting residues with each protein. The twinfilin–actin structure is used here as a model for the cofilin–actin interactions. Protein interfaces were identified in CONTACT (Winn et al., 2011) and the figure produced in Jalview (Waterhouse et al., 2009).]
(Goodson et al., 1996) and tropomyosins (Balasubramanian et al., 1992) all show lethality in one or more yeast species (Costanzo et al., 2010; Moseley and Goode, 2006).

The two human cytoplasmic actins (β and γ) and four human muscle actins (α-cardiac, α-skeletal, α-smooth and γ-smooth) are extremely well conserved in sequence despite the muscle isoforms having to fulfill the highly specialized function of muscle contraction (Fig. 1; supplementary material Table S1). Nevertheless, comparison of vertebrate actins shows that there is a high level of conservation of the differences between the muscle and cytoskeletal isoforms (Fig. 3), which is not seen between the bacterial actins, MreB and FtsA (Fig. 1). Indeed, the amino acid sequences of the six avian and

![Fig. 3. Phylogenetic relationships of actins between diverse animal species. Cytoplasmic and muscle actin isoforms are highly conserved between species. Actin protein sequences from human (H. sapiens), mouse (M. musculus), chicken (G. gallus), frog (X. tropicalis), zebrafish (D. rerio), cod (G. morhua), opossum (M. domestica) and lizard (A. carolinensis) are compared in a maximum likelihood phylogenetic tree. Sequences within the pink box indicate those found in the skeletal and smooth muscle actin classes and those within the blue box indicate those found within the cytoplasmic class. ACTA, ACTC, ACTH and ACTS refer to skeletal, cardiac, aortic smooth and γ-enteric smooth muscle actins, respectively. ACTB and ACTG are the cytoplasmic β- and γ-actin, respectively. Asterisks highlight the fish actins that show different patterns of isoform conservation. The tree-wide SR is 0.050, which breaks down into 0.020 and 0.019, for cytoplasmic and muscle actins, respectively. In comparison, the average substitutions per base (SB) in the equivalent DNA phylogenetic tree (not shown) is 0.47, indicating the restrictive pressure at the protein level.](image-url)
mammalian actins are almost invariant for each isoform. This indicates that the selection pressure that has maintained actin sequence conservation has tolerated only a small degree of variation, which is under an even greater degree of selection pressure. This reflects the lack of functional redundancy of these isoforms (Schevzov et al., 1992), which have important functional consequences in terms of their interactions with specific actin-binding proteins and location to specific structures (Dugina et al., 2009; Perrin and Ervasti, 2010).

Muscle actins are likely to have experienced different selection pressures, in comparison to cytoplasmic actins, that have led to the optimization of their sequences for muscle contraction. So why have they diverged so little? In muscle cells, the usual cytoplasmic actin functions take place alongside the muscle-specific actin functions. Transgenic expression of γ-actin leads to substantial incorporation of γ-actin into the thin filaments (Jaeger et al., 2009), suggesting that some crossover of muscle actins and cytoplasmic actins can be tolerated. Indeed, biochemists routinely use rabbit skeletal muscle actin to characterize cytoplasmic actin-regulating proteins in vitro. Thus we conjecture that muscle actins may have been subject to selection pressure, as drastic changes would lead to the impairment of normal cytoplasmic actin function through cross-contamination of isoforms. In other words, animal muscle actins may have experienced a high degree of negative selection pressure despite their specialization in function.

Thus eukaryotic actin, together with its repertoire of regulatory proteins, comprises an exquisitely conserved, universal force-generating polymerizing machine that is integrated into many biological processes. The actin polymerization machine is well conserved from single cellular eukaryotes, such as yeast, to multicellular organisms, such as man, which represents a period of about one billion years (Figs 1 and 2). In the background of rapidly evolving genomes, we next consider how the actin system might have evolved during the multicellularization process to allow for the expanding need of actin participation in an increasing number of processes, many of which are cell-type specific.

In all the multicellular organisms that are analyzed here, actin force generation appears to have been incorporated into an increased number of processes by enlarging the range of filament-nucleating complexes. For example, humans have many more formins than yeasts (Table 1). Furthermore, complicated actin filament geometries, which are comprised of protein-mediated crosslinked filaments, are found in filopodia, stress fibers, endocytotic structures, the contractile ring and root tips. The differential regulation of actin-filament side-binding proteins that are necessary for setting up defined actin geometries, and the regulation of filament lifetimes in each structure, appear to have evolved differently between eukaryotes and the plants, which we outline below.

### Table 1. The numbers of genes with distinct protein products by species

<table>
<thead>
<tr>
<th>Species</th>
<th>Muscle actin</th>
<th>Cytoplasmic actin</th>
<th>ADF</th>
<th>Profilin</th>
<th>Formin</th>
<th>Tmod or Lmod</th>
<th>Tm</th>
<th>Tm isoforms</th>
<th>Tubulin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metazoa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homo sapiens</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>13</td>
<td>7</td>
<td>4</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>Gallus gallus</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>11</td>
<td>7</td>
<td>4</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>Xenopus tropicalis</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>12</td>
<td>7</td>
<td>4</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td>Danio rerio</td>
<td>10</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>26</td>
<td>8</td>
<td>4</td>
<td>(6)</td>
<td>48</td>
</tr>
<tr>
<td>Ciona intestinalis</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>0</td>
<td>3</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Drosophila melanogaster</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>22</td>
<td>7</td>
</tr>
<tr>
<td>Caenorhabditis elegans</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td><strong>Plants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td>–</td>
<td>10</td>
<td>12</td>
<td>5</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>17</td>
</tr>
<tr>
<td>Musa acuminata</td>
<td>–</td>
<td>12</td>
<td>27</td>
<td>13</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>34</td>
</tr>
<tr>
<td>Glycine max</td>
<td>–</td>
<td>17</td>
<td>18</td>
<td>8</td>
<td>37</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>37</td>
</tr>
<tr>
<td>Brassica rapa</td>
<td>–</td>
<td>11</td>
<td>17</td>
<td>13</td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>29</td>
</tr>
<tr>
<td>Chlamydomonas reinhardtii</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>7</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Neurospora crassa</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Aspergillus nidulans</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Schizosaccharomyces pombe</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td><strong>Protists</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entamoeba histolytica</td>
<td>–</td>
<td>1 (7)</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
<td>Phaeodactylus tricornutum</td>
<td>–</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>Trypanosoma brucei</td>
<td>–</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>11</td>
</tr>
<tr>
<td>Dictyostelium discoideum</td>
<td>–</td>
<td>8 (24)</td>
<td>8</td>
<td>3</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>4</td>
</tr>
</tbody>
</table>

Lmod, leiomodin; Tmod, tropomodulin; Tm, tropomyosin. Multicellular plants have high numbers of actin, profilin and cofilin isoforms with no tropomyosins. Metazoa and fungi have fewer actin, profilin and cofilin isoforms but have evolved to have tropomyosins. Numbers in brackets signify the total number of genes, where two or more genes encode identical protein products. Archaia generally do not possess actin. The Tm column shows the total number of isoforms of tropomyosin including known alternative splicing products.
multicellular plants have ten or more actsins, with many having a substantially larger number. For example, *Arabidopsis thaliana* has 10 actin genes and *Glycine max* (soybean) has 17 (Table 1). Evolutionary analysis of the plant actsins reveals that there are isoforms that are often more closely related to those of other species than to isoforms within the same species. For example, comparison of *Arabidopsis* and soybean actsins reveals that many subgroups of *Arabidopsis* actsins are interspersed among the soybean actsins (Fig. 4). Thus, plant actsins do not show the same high level of isoform conservation that is observed for animal actsins (Fig. 3).

Phylogenetic evidence reveals two mechanisms underlying the expansion of actin genes in plants. Firstly, like MreB and FtsA from bacteria (and also like metazoa, see below), specific plant actin isoforms have been conserved between species. In other words, there are isoforms that have been under negative selection pressure between species (Fig. 4). This is most compatible with a conserved biological function for these isoforms, which has restricted the tolerated changes in amino acids over hundreds of millions of years. Secondly, there is also clear evidence of actin gene expansion within species. This is typified in the soybean gene family where actin genes within a subgroup are generally more highly related to each other than the genes from *Arabidopsis* (Fig. 4). This pattern of relationships is consistent with a model in which the evolution of a species is accompanied by multiple rounds of gene duplication to create an expanded gene family, which in plants, happened in part by a series of genome duplications (Lee et al., 2013; Lynch and Conery, 2000).

We propose that plant actsins have a range of diverged functional properties based on the observation that within a plant species, the divergence between individual gene products is substantially greater that that seen in animals (An et al., 1996a; An et al., 1996b; Huang et al., 1997; Meagher et al., 1999). For example, the divergence of the human actsins [substitutions per residue (SR)=0.01, Fig. 3] is smaller than that seen between the *Arabidopsis* actsins (SR=0.09, Fig. 4). One significant observation is that the divergence in amino acid sequence in plant actsins is more frequently associated with surface residues than that observed with animal actsins (Kandasamy et al., 2007). This might be because there have been changes in the nature of interactions between the different plant actsins and actin-binding proteins. In turn, this poses the question of why plants have greater divergence of their actsins than vertebrates. Although it is possible that plants have a greater requirement for highly specialized actin filaments than vertebrates, we propose that it is not the actin itself that provides the greatest filament specialization in vertebrates (see below).

There are ten *Arabidopsis* actin genes, which display different expression patterns and are implicated in a range of cell functions (Slájcherová et al., 2012). The eight expressed genes can be divided into two classes based on the cell types in which they are expressed, vegetative and reproductive, and the genes in each class are more closely related to each other than to the other class (Fig. 4) (Meagher et al., 1999). These two classes diverged about 400 million years ago. Induction of high levels of expression of a reproductive class of actin in vegetative tissue led to dwarfing of plants and disrupted cytoskeleton architecture, whereas similar expression of a vegetative actin had no effect. This result could be accounted for by isoform-specific differences in the interaction of actsins with actin-binding proteins. Co-expression of both reproductive actin and actin-binding protein isoforms in vegetative tissue resulted in normal growth and eliminated the impact of expression of the reproductive actin alone (Kandasamy et al., 2007). This indicates the existence of co-evolution of actin and actin-binding protein isoforms, which perform specialized functions in a specific cellular context.

A comparison of the numbers of actin genes with the numbers of genes encoding actin-binding proteins in plants is compatible with preferential interactions between specific actin isoforms and specific actin-binding protein isoforms (Table 1). For example, *Arabidopsis* has ten actsins, 12 actin-depolymerizing factors (ADFs) and five profilins, whereas humans have only two cytoplasmic actsins, three ADFs and three profilins. Plants are known to have larger gene numbers than metazoa, with *Arabidopsis thaliana*, *Glycine max* and humans having ~27,400, 56,000 and 19,000 genes, respectively. These large numbers of plant genes have arisen from genome duplications. However, the plant genome sizes are not sufficient to attribute the even more highly elevated numbers of actsins and actin-binding proteins to genome duplications (Lee et al., 2013).

A simple explanation for the diversity of plant actsins might be provided by the expanded numbers of forms (Table 1) – if different forms are able to discriminate between different actsins, possibly through recruiting dedicated profilins, then we hypothesize that each form might be able to control the formation of homopolymers of different actin isoforms. If this is true, each type of actin might have co-evolved with a corresponding set of...
actin-binding proteins, such as a preferred profilin and cofilin based on similar isoform numbers in each plant (Table 1). Thus, plant cells might be capable of making multiple distinct and functionally specialized actin filament systems (Kandasamy et al., 2007). This would parallel the strategy that evolved in bacteria as discussed above.

Integration of tropomyosins into actin filaments

Perhaps the most surprising observation of actin filament evolution is that only one or two actin isoforms are required to perform the wide array of functions required of cytoplasmic actin in fungi and metazoan (Table 1). We hypothesize that the introduction of tropomyosin into the actin filaments of fungi and metazoans provided a simple mechanism to diversify the functional capacity of actin filaments in these kingdoms without expanding the number of actin isoforms (Gunning et al., 2008). Tropomyosin forms two continuous co-polymers with actin that lie along the major grooves on either side of the actin filament (Phillips et al., 1979). Fission yeast, which has one actin and one tropomyosin gene, generates three compositionally distinct types of actin filaments: one class of actin filaments without tropomyosin (Kovar et al., 2011), a second type containing a co-polymer of actin with N-terminal acetylated tropomyosin and a third comprising a co-polymer of actin with non-acetylated tropomyosin (Coulton et al., 2010). These three classes of actin filaments are spatially segregated in the cell and the two tropomyosin-containing filaments are able to discriminate between and segregate different myosin motors (Clayton et al., 2010; Coulton et al., 2010). This tropomyosin-based selectivity is important because, to our knowledge, all myosins that have been isolated from yeast, fungi and plants productively interact with mammalian actin filaments containing just skeletal muscle actin.

Recent data indicate that tropomyosin regulates the functional capabilities of the MyoV motor (Hodges et al., 2012). The increasing complexity of metazoans is paralleled by increasing numbers of tropomyosin genes and larger numbers of alternatively-spliced isoforms. Phylogenetic analysis of the tropomyosin genes shows that they are under a high degree of selection pressure and this is most dramatically seen in the vertebrate tropomyosins (Barua et al., 2011; Ochiai et al., 2010) (Fig. 5). The four vertebrate genes have undergone limited changes over the last 500 million years (Schevzov et al., 2011) and, moreover, the specific vertebrate genes are highly conserved suggesting that the entire surface of the coiled-coil is under strong selection pressure (Schevzov et al., 2011). The expansion of the number of tropomyosin isoforms, rather than of actin isoforms, that accompanies the vertebrate radiation leads us to hypothesize that it is the compositional diversity of actin filaments that has been the subject of selection (Table 1).

It is an axiom of evolution that if you spatially segregate isoforms you will inevitably select for specialized function that is based on the spatial context of isoform location (Gunning, 2003). It is therefore not surprising that the tropomyosin isoforms of mammals have acquired specialized functions. For instance, cytoplasmic isoforms of tropomyosin have been shown to be functionally distinct in mice (Hook et al., 2004; Hook et al., 2011). This functional specialization of tropomyosin was first suggested by the tropomyosin-isoform-dependent protection of actin filaments from gelsolin-mediated severing (Ishikawa et al., 1989a; Ishikawa et al., 1989b; Nag et al., 2013). Subsequent studies have shown that different tropomyosin isoforms allow actin filaments to functionally discriminate between myosins (Bryce et al., 2003; Fanning et al., 1994; Tang and Ostap, 2001). Tropomyosin allosterically increases the hydrophobic and stereospecific interactions between myosin and actin, providing a possible basis for recruitment and selection of particular myosins (Lu et al., 2006). Furthermore, post-translational acetylation and phosphorylation of mammalian tropomyosin increases the variation in the system through altering the association with actin and changing actin–myosin dynamics, respectively (Hitchcock-DeGregori and Heald, 1987; Nixon et al., 2013). Tropomyosins also respond
differently to ADFs and cofilins (Bryce et al., 2003), and interact with specific tropomodulins and leiomodins, proteins that cap the pointed end of an actin-tropomyosin co-polymer and influence the filament dynamics and lifetimes (Yamashiro et al., 2012) and other actin-binding proteins (Creed et al., 2011; Kostyukova and Hitchcock-DeGregori, 2004; Sung and Lin, 1994; Watakabe et al., 1996).

Perhaps the most dramatic specialization of the actin filament is found in muscle. The ability to form contractile tissue came from specialization of the cytoskeleton. The principle of contractile force generated by the interaction of myosin II motors with actin–tropomyosin co-polymers is as ancient as the yeast contractile ring. The mechanism by which these compositionally distinct filaments, in terms of their tropomyosin content, are generated in fission yeast has recently been identified (Johnson et al., 2014). The two fission yeast formins generate actin filaments with different tropomyosin isoform compositions, and hence, with different functional properties. Manipulation of a formin to a new location in the cell led to the assembly of actin filaments complete with the formin-specific tropomyosin at the new site (Johnson et al., 2014).

Comparisons of the mammalian actin and tropomyosin sequences with those of tubulins and intermediate filaments are revealing. There are many more tubulin genes (24 in humans) than actin genes (Table 1) and they show a distribution in sequence identities that ranges from almost identical isotypes to those that show relatively more diversity in sequence than mammalian actins (Ludueña, 2013). Several of the tubulin isotypes have been implicated in discrete processes. Thus tubulins would appear to fit with the model that we have suggested for plants, whereby the number of genes have expanded and diversified in a background of negative selection pressure. That negative selection probably arises from a common set of interacting proteins. The sequence differences between tubulins allowed extension of function through the acquisition of sets of unique interactions. The mammalian intermediate filament gene family has also expanded, particularly among the keratins of which there are 54 in humans. The selection of keratins that are expressed changes with epithelial cell type, differentiation state and developmental stage (Chu and Weiss, 2002). This pattern has parallels with the prokaryotic one-filament-one-function model, whereby keratin genes have expanded to produce independent systems that have varying properties. Thus, a mammalian cell expresses the subset of keratins that meets its requirements.

We propose that the compositional diversity of actin filaments in terms of the actin and tropomyosin (in the case of fungi and metazoa) isoforms they contain, has provided an extraordinary diversity of function. Spatial and temporal segregation of both actin and tropomyosin isoforms provided the context in which to specialize. As cells became more specialized and architecturally complex, the actin, and subsequently tropomyosin, isoforms provided the opportunity to independently regulate a range of actin filament functions.

Conclusions
Organisms, from bacteria to plants and man, use multiple acts to conduct the numerous functions demanded of this polymer system. In general, the acts appear to mostly form homopolymers, which provide fidelity of function to each polymer. The ability to include force and form into an expanding number of biological processes during eukaryotic evolution fashioned actin as a universal polymerization machine. Once created, we hypothesize that the central player actin was ‘frozen’ in evolutionary time due to negative selection imposed by its involvement in a multitude of functionally crucial processes. Thus, the inherent structural and dynamic characteristics of the actin filament system will have shaped its own evolution. The ‘one-filament-one-function’ system in bacteria was replaced by a ‘universal-actin-pool’ in eukaryotes which could be tapped into by many processes. This communal approach probably encountered challenges, particularly during the specialization of intracellular space in cells and in moving from single cellular to multicellular organisms, resulting in the expansion in the available variety of actin filaments. Different branches of eukaryotes appear to have evolved distinct methods to do this. Plants have an expanded number of actin genes allowing subtle variations in the actin produced, whereas tropomyosins have evolved in animals, which can differentially regulate the interactions with the filaments. In eukaryotes, post-translational modifications provide a further level in variety of actin filaments (Terman and Kashina, 2013). These adaptations have expanded the repertoire of actin function in the multicellular environment. However, the principle first established for bacteria of a specialized function for each type of compositionally distinct actin filament can be applied to understanding actin filaments across the kingdoms.

Acknowledgements
We would like to thank Byrappa Venkatesh and Swaine Chen for helpful discussions. P.G. thanks Jeff Hook for help with data management.

Competing interests
Peter Gunning is a Director of Novogen Ltd, which is commercialising anti-tropomyosin drugs.

Funding
U.G., D.P. and R.C.R. thank A*STAR for support. P.G. is supported by The Kid’s Cancer Project; and the National Health and Medical Research Council (NHMRC) [grant number APPS10762].

Supplementary material
Supplementary material available online at http://jcs.biologists.org/lookup/suppl/doi:10.1242/jcs.165563/-/DC1

References
Formins direct Arp2/3-independent actin filament assembly to polarize cell growth and to recruit of actin-binding proteins to actin filaments. Exp. Cell Res. 317, 249-261.


HYPOTHESIS


<table>
<thead>
<tr>
<th>Name</th>
<th>UniProt ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>MreB_B.vietnamiensis</td>
<td>A4JJ52_BURVG</td>
</tr>
<tr>
<td>MreB2_B.vietnamiensis</td>
<td>A4JIT2_BURVG</td>
</tr>
<tr>
<td>MreB_M.thermoacetica</td>
<td>Q2RL22_MOOTA</td>
</tr>
<tr>
<td>MreB2_M.thermoacetica</td>
<td>Q2RFY6_MOOTA</td>
</tr>
<tr>
<td>MreB_N.thermophilus</td>
<td>B2A3F3_NATTJ</td>
</tr>
<tr>
<td>MreB2_N.thermophilus</td>
<td>B2A6A0_NATTJ</td>
</tr>
<tr>
<td>MreB_V.cholerae</td>
<td>H9L4S9_VIBCH</td>
</tr>
<tr>
<td>MreB_S.aciditrophicus</td>
<td>Q2LQZ5_SYNAS</td>
</tr>
<tr>
<td>MreB_C.tetani</td>
<td>Q892M2_CLOTE</td>
</tr>
<tr>
<td>MreB2_C.tetani</td>
<td>Q898X0_CLOTE</td>
</tr>
<tr>
<td>MreB_E.coli</td>
<td>P0A9X4_ECOLI</td>
</tr>
<tr>
<td>FtsA_E.coli</td>
<td>P0ABH0_ECOLI</td>
</tr>
<tr>
<td>FtsA_B.vietnamiensis</td>
<td>A4JB98_BURVG</td>
</tr>
<tr>
<td>FtsA_M.thermoacetica</td>
<td>Q2RK73_MOOTA</td>
</tr>
<tr>
<td>FtsA_N.thermophilus</td>
<td>B2A2H5_NATTJ</td>
</tr>
<tr>
<td>FtsA_V.cholerae</td>
<td>Q9KPH0_VIBCH</td>
</tr>
<tr>
<td>FtsA_S.aciditrophicus</td>
<td>Q2LR54_SYNAS</td>
</tr>
<tr>
<td>FtsA_C.tetani</td>
<td>Q895Z1_CLOTE</td>
</tr>
<tr>
<td>ParM_N.thermophilus</td>
<td>B2A2C2_NATTJ</td>
</tr>
<tr>
<td>ParM_S.aciditrophicus</td>
<td>Q2LTX1_SYNAS</td>
</tr>
<tr>
<td>ParM_B.vietnamiensis</td>
<td>A4JVG1_BURVG</td>
</tr>
<tr>
<td>ParM2_B.vietnamiensis</td>
<td>A4JTY5_BURVG</td>
</tr>
<tr>
<td>ParM_C.tetani</td>
<td>Q89A01_CLOTE</td>
</tr>
<tr>
<td>ParM_M.thermoacetica</td>
<td>Q2RLL0_MOOTA</td>
</tr>
<tr>
<td>ParM_V.cholerae</td>
<td>Q7WZH8_VIBCL</td>
</tr>
<tr>
<td>ParM_K.pneumoniae</td>
<td>G8XCL7_KLEPH</td>
</tr>
<tr>
<td>ParM2_K.pneumoniae</td>
<td>G8W576_KLEPH</td>
</tr>
<tr>
<td>ParM_S.dysenteriae</td>
<td>Q327C0_SHIDS</td>
</tr>
<tr>
<td>ParM_S.typhimurium</td>
<td>E8XLQ8_SALT4</td>
</tr>
<tr>
<td>MreB_S.typhimurium</td>
<td>E8XD78_SALT4</td>
</tr>
<tr>
<td>MreB_K.pneumoniae</td>
<td>G8W1F2_KLEPH</td>
</tr>
<tr>
<td>MreB_S.dysenteriae</td>
<td>Q32B89_SHIDS</td>
</tr>
<tr>
<td>FtsA_S.typhimurium</td>
<td>E8XH43_SALT4</td>
</tr>
<tr>
<td>FtsA_K.pneumoniae</td>
<td>G8VWK7_KLEPH</td>
</tr>
<tr>
<td>FtsA_S.dysenteriae</td>
<td>Q32JZ8_SHIDS</td>
</tr>
<tr>
<td>ACTB_H.sapiens</td>
<td>ACTB_HUMAN</td>
</tr>
<tr>
<td>ACTC_H.sapiens</td>
<td>ACTC_HUMAN</td>
</tr>
<tr>
<td>ACTA_H.sapiens</td>
<td>ACTA_HUMAN</td>
</tr>
<tr>
<td>ACTG_H.sapiens</td>
<td>ACTG_HUMAN</td>
</tr>
<tr>
<td>ACTH_H.sapiens</td>
<td>ACTH_HUMAN</td>
</tr>
<tr>
<td>ACTS_H.sapiens</td>
<td>ACTS_HUMAN</td>
</tr>
<tr>
<td>ACT_S.pombe</td>
<td>ACT_SCHPO</td>
</tr>
</tbody>
</table>
The accession codes for the proteins used in Fig. 3.

Nomenclature according to UniProt:
ACTA - aortic smooth muscle
ACTB - beta
ACTC - cardiac muscle
ACTG - gamma
ACTH - gamma enteric smooth muscle
ACTS - skeletal muscle

<table>
<thead>
<tr>
<th>Name_species</th>
<th>ID</th>
<th>Database Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTA_A.carolinensis</td>
<td>ENSACAP00000000525</td>
<td>alpha 2, smooth muscle, aorta</td>
</tr>
<tr>
<td>ACTB_A.carolinensis</td>
<td>327288208</td>
<td>cytoplasmic type 5</td>
</tr>
<tr>
<td>ACTC_A.carolinensis</td>
<td>ENSACAP00000001039</td>
<td>Uncharacterized protein, gene:actc1</td>
</tr>
<tr>
<td>ACTCL_A.carolinensis</td>
<td>ENSACAP0000012230</td>
<td>Uncharacterized protein</td>
</tr>
<tr>
<td>ACTG_A.carolinensis</td>
<td>ENSACAP0000014970</td>
<td>gamma 1</td>
</tr>
<tr>
<td>ACTHL_A.carolinensis</td>
<td>ENSACAP000001688</td>
<td>gamma 2, smooth muscle, enteric</td>
</tr>
<tr>
<td>ACTH1_A.carolinensis</td>
<td>ENSACAP0000013677</td>
<td>Uncharacterized protein</td>
</tr>
<tr>
<td>ACTS_A.carolinensis</td>
<td>ENSACAP0000000779</td>
<td>Uncharacterized protein, gene:acta1</td>
</tr>
<tr>
<td>ACTA1a_D.rerio</td>
<td>49901223</td>
<td>alpha 2, smooth muscle, aorta</td>
</tr>
<tr>
<td>ACTA1b_D.rerio</td>
<td>ENA AAQ97738</td>
<td>alpha 2, smooth muscle, aorta</td>
</tr>
<tr>
<td>ACTB1a_D.rerio</td>
<td>ENSDARG00000037746</td>
<td>beta 1</td>
</tr>
<tr>
<td>ACTB1b_D.rerio</td>
<td>ENSDARG00000037870</td>
<td>beta 2</td>
</tr>
<tr>
<td>ACTC1a1_D.rerio</td>
<td>ENSDARG00000057911</td>
<td>alpha, cardiac muscle 1</td>
</tr>
<tr>
<td>ACTC1a2_D.rerio</td>
<td>ENSDARG00000076126</td>
<td>alpha, cardiac muscle 1</td>
</tr>
<tr>
<td>ACTC1a3_D.rerio</td>
<td>ENSDARG00000042535</td>
<td>alpha, cardiac muscle 1a</td>
</tr>
<tr>
<td>ACTC1a4_D.rerio</td>
<td>ENSDARG00000037840</td>
<td>alpha, cardiac muscle 1b</td>
</tr>
<tr>
<td>ACTS1a1_D.rerio</td>
<td>ENSDARG00000036371</td>
<td>alpha 1a, skeletal muscle</td>
</tr>
<tr>
<td>ACTS1a2_D.rerio</td>
<td>ENA AAH93200</td>
<td>alpha 1a, skeletal muscle</td>
</tr>
<tr>
<td>ACTS1b1_D.rerio</td>
<td>ENSDARG00000055618</td>
<td>alpha 1b, skeletal muscle</td>
</tr>
<tr>
<td>ACTS1b2_D.rerio</td>
<td>ENA AAH98546</td>
<td>alpha 1b, skeletal muscle</td>
</tr>
<tr>
<td>ACTA_G.gallus</td>
<td>ENSGALG00000006343</td>
<td>alpha 2, smooth muscle, aorta</td>
</tr>
<tr>
<td>ACTB_G.gallus</td>
<td>ENSGALG00000009621</td>
<td>beta</td>
</tr>
<tr>
<td>ACTC_G.gallus</td>
<td>ENSGALG00000009844</td>
<td>alpha, cardiac muscle 1 (ACTC1)</td>
</tr>
<tr>
<td>ACTG_G.gallus</td>
<td>ENSGALG00000028749</td>
<td>ACTG1, cytoplasmic 2 Actin, cytoplasmic 2</td>
</tr>
<tr>
<td>ACTH_G.gallus</td>
<td>ENA AAB27386</td>
<td>gamma-enteric smooth muscle</td>
</tr>
<tr>
<td>ACTS_G.gallus</td>
<td>ENSGALG0000011086</td>
<td>alpha 1, skeletal muscle (ACTA1)</td>
</tr>
<tr>
<td>ACTB_G.morhua</td>
<td>ENSGMOP00000008161</td>
<td>Beta-actin</td>
</tr>
<tr>
<td>ACTB1L_G.morhua</td>
<td>ENSGMOP0000001711</td>
<td>Beta-actin</td>
</tr>
<tr>
<td>ACTG_G.morhua</td>
<td>ENSGMOP0000010348</td>
<td>Beta-actin</td>
</tr>
<tr>
<td>ACTC1_G.morhua</td>
<td>ENSGMOP0000012126</td>
<td>alpha, cardiac muscle 1</td>
</tr>
<tr>
<td>ACTC2_G.morhua</td>
<td>ENSGMOP0000005683</td>
<td>alpha, cardiac muscle 1</td>
</tr>
<tr>
<td>ACTC3_G.morhua</td>
<td>ENSGMOP0000013330</td>
<td>alpha, cardiac muscle 1</td>
</tr>
<tr>
<td>ACTC4_G.morhua</td>
<td>ENSGMOP000001484</td>
<td>alpha, cardiac muscle 1</td>
</tr>
<tr>
<td>ACTA_H.sapiens</td>
<td>ENSG00000107796</td>
<td>aortic smooth muscle</td>
</tr>
<tr>
<td>ACTB_H.sapiens</td>
<td>ENSG00000075624</td>
<td>beta</td>
</tr>
<tr>
<td>ACTC_H.sapiens</td>
<td>ENSG00000159251</td>
<td>cardiac muscle</td>
</tr>
<tr>
<td>Gene Name</td>
<td>Accession Number</td>
<td>Description</td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>ACTG_H.sapiens</td>
<td>ENSG000000184009</td>
<td>gamma</td>
</tr>
<tr>
<td>ACTH_H.sapiens</td>
<td>ENSG00000163017</td>
<td>gamma enteric smooth muscle</td>
</tr>
<tr>
<td>ACTS_H.sapiens</td>
<td>ENSG00000143632</td>
<td>skeletal muscle</td>
</tr>
<tr>
<td>ACTB_M.domestica</td>
<td>ENSMODG00000023940</td>
<td>Uncharacterized protein.</td>
</tr>
<tr>
<td>ACTC_M.domestica</td>
<td>ENSMODG0000001384</td>
<td>alpha, cardiac muscle 1</td>
</tr>
<tr>
<td>ACTG_M.domestica</td>
<td>ENSMODG0000002647</td>
<td>gamma 1</td>
</tr>
<tr>
<td>ACTH1a_M.domestica</td>
<td>334313528</td>
<td>gamma-enteric smooth muscle X2</td>
</tr>
<tr>
<td>ACTH1b_M.domestica</td>
<td>ENSMODG0000010521</td>
<td>gamma 2, smooth muscle, enteric</td>
</tr>
<tr>
<td>ACTS_M.domestica</td>
<td>334322342</td>
<td>alpha skeletal muscle</td>
</tr>
<tr>
<td>ACTA_M.musculus</td>
<td>ENSMUSG00000035783</td>
<td>aortic smooth muscle</td>
</tr>
<tr>
<td>ACTB_M.musculus</td>
<td>ENSMUSG00000029580</td>
<td>beta</td>
</tr>
<tr>
<td>ACTC_M.musculus</td>
<td>ENSMUSG00000068614</td>
<td>cardiac muscle</td>
</tr>
<tr>
<td>ACTG_M.musculus</td>
<td>ENSMUSG00000062825</td>
<td>gamma</td>
</tr>
<tr>
<td>ACTH_M.musculus</td>
<td>ENSMUSG00000059430</td>
<td>gamma enteric smooth muscle</td>
</tr>
<tr>
<td>ACTS_M.musculus</td>
<td>ENSMUSG00000031972</td>
<td>skeletal muscle</td>
</tr>
<tr>
<td>ACTA1a_X.tropicalis</td>
<td>ENSXETG00000009279</td>
<td>alpha 2, smooth muscle, aorta</td>
</tr>
<tr>
<td>ACTA1b_X.tropicalis</td>
<td>ENSXETP00000020391</td>
<td>alpha 2, smooth muscle, aorta</td>
</tr>
<tr>
<td>ACTB_X.tropicalis</td>
<td>ENSXETG00000025116</td>
<td>beta</td>
</tr>
<tr>
<td>ACTC_X.tropicalis</td>
<td>ENSXETG00000012911</td>
<td>alpha, cardiac muscle 1</td>
</tr>
<tr>
<td>ACTG_X.tropicalis</td>
<td>ENSXETG00000027641</td>
<td>gamma 1</td>
</tr>
<tr>
<td>ACTS_X.tropicalis</td>
<td>ENSXETG00000021417</td>
<td>alpha 1, skeletal muscle</td>
</tr>
</tbody>
</table>
The accession codes for the tropomyosins in Fig. 5.

TPM_S.cerevisiae  YNL079C

TPM1_X.tropicalis  ENSXETP00000021409-1
TPM2_X.tropicalis  ENSXETP00000021198-2
TPM3_X.tropicalis  ENSXETP00000056558-3
TPM4_X.tropicalis  ENSXETP00000019155-4

TPM1_H.sapiens  ENSP00000351022-1
TPM2_H.sapiens  ENSP00000367542-2
TPM3_H.sapiens  ENSP00000357516-3
TPM4_H.sapiens  ENSP00000439135-4

TPM1_M.musculus  ENSMUSP00000109337-1
TPM2_M.musculus  ENSMUSP00000103546-2
TPM3_M.musculus  ENSMUSP00000062920-3
TPM4_M.musculus  ENSMUSP0000003575-4

TPM1_G.gallus  ENSGALP00000005562-1
TPM2_G.gallus  ENSGALP00000044062-2
TPM3_G.gallus  ENSGALP00000022004-3
TPM4_G.gallus  ENSGALP00000038490-4

TPM1_M.domestica  ENSMODP00000018611-1
TPM2_M.domestica  ENSMODP0000009260-2
TPM3_M.domestica  ENSMODP00000021531-3
TPM4_M.domestica  ENSMODP00000018612-4

TPM2_A.carolinensis  ENSACAG00000010881-2
TPM4_A.carolinensis  ENSACAG00000008516-4

TPM1_D.rerio  ENSDARP0000002483-1
TPM2_D.rerio  ENSDARP00000089645-2
TPM3_D.rerio  ENSDARP00000039656-3
TPM4_D.rerio  ENSDARP00000088646-4

TPM1_G.morhua  ENSGMOG0000006834-1
TPM1a_G.morhua  ENSGMOG0000006759-1a
TPM2_G.morhua  ENSGMOG0000013266-2
TPM3_G.morhua  ENSGMOG0000017182-3
TPM3a_G.morhua  ENSGMOG000004551-3a
TPM4_G.morhua  ENSGMOG0000015606-4
TPM4a_G.morhua  ENSGMOG0000012492-4a
### Accession numbers for Table 1

#### ACTINS

**Homo_sapiens** 6  *Cytoplasmic* 2
ACTG ENSG00000184009  ACTB ENSG00000075624;  *Muscle* 4
ACTA ENSG00000107796  ACTH ENSG00000163017

**Gallus_gallus** 6  *Cytoplasmic* 2
ACTG ENSGALG00000028749  ACTB ENSGALG00000009844

**Xenopus_tropicalis** 6  *Cytoplasmic* 2
ACTG ENSXETG00000027641

**Danio_rerio** 11  *Cytoplasmic* 2
ACTG ENSDARG00000037870

**Ciona_intestinalis** 11

**Arabidopsis_thaliana** 10
AT2G42090  AT3G18780  AT5G09810

**Musa_acuminata** 12
GSMUA_Achr1P05990  GSMUA_Achr3P02960  GSMUA_Achr7P10410

**Glycine_max** 17
GLYMA15G05570  GLYMA08G15480  GLYMA05G32220

**Brassica_rapa** 11
Bra006033  Bra028615  Bra034778

**Chlamydomonas_reinhardtii** 1  *EDO98923*

**Saccharomyces_cerevisiae** 1  YFL039C

**Neurospora_crassa** 1  EFNCRG00000038931

**Aspergillus_nidulans** 1  CADANIAG00007315

**Schizosaccharomyces_pombe** 1  SPBC32H8.12c1

**Entamoeba_histolytica** 1  (7)
rna_EHI_182900  rna_EHI_142730  rna_EHI_107290

**Phaeodactylum_tricornutum** 3  Phatr51157  Phatr29812  Phatr29136

**Dictyostelium_discoideum** 8  (24)
DDB0220444  DDB0185129  DDB0185127  DDB0185015

<table>
<thead>
<tr>
<th>Organism</th>
<th>Accession Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Homo_sapiens</strong></td>
<td>ACTG: ENSG00000184009, ACTB: ENSG00000075624, ACTA: ENSG00000107796, ACTH: ENSG00000163017</td>
</tr>
<tr>
<td><strong>Gallus_gallus</strong></td>
<td>ACTG: ENSGALG00000028749, ACTB: ENSGALG00000009844</td>
</tr>
<tr>
<td><strong>Xenopus_tropicalis</strong></td>
<td>ACTG: ENSXETG00000027641</td>
</tr>
<tr>
<td><strong>Danio_rerio</strong></td>
<td>ACTG: ENSDARG00000037870</td>
</tr>
<tr>
<td><strong>Ciona_intestinalis</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Arabidopsis_thaliana</strong></td>
<td>AT2G42090, AT3G18780, AT5G09810</td>
</tr>
<tr>
<td><strong>Musa_acuminata</strong></td>
<td>GSMUA_Achr1P05990, GSMUA_Achr3P02960, GSMUA_Achr7P10410</td>
</tr>
<tr>
<td><strong>Glycine_max</strong></td>
<td>GLYMA15G05570, GLYMA08G15480, GLYMA05G32220</td>
</tr>
<tr>
<td><strong>Brassica_rapa</strong></td>
<td>Bra006033, Bra028615, Bra034778</td>
</tr>
<tr>
<td><strong>Chlamydomonas_reinhardtii</strong></td>
<td>EDO98923</td>
</tr>
<tr>
<td><strong>Saccharomyces_cerevisiae</strong></td>
<td>YFL039C</td>
</tr>
<tr>
<td><strong>Neurospora_crassa</strong></td>
<td>EFNCRG00000038931</td>
</tr>
<tr>
<td><strong>Aspergillus_nidulans</strong></td>
<td>CADANIAG00007315</td>
</tr>
<tr>
<td><strong>Schizosaccharomyces_pombe</strong></td>
<td>SPBC32H8.12c1</td>
</tr>
<tr>
<td><strong>Entamoeba_histolytica</strong></td>
<td>(7) rna_EHI_182900, rna_EHI_142730, rna_EHI_107290</td>
</tr>
<tr>
<td><strong>Phaeodactylum_tricornutum</strong></td>
<td>Phatr51157, Phatr29812, Phatr29136</td>
</tr>
<tr>
<td><strong>Dictyostelium_discoideum</strong></td>
<td>(24) DDB0220444, DDB0185129, DDB0185127, DDB0185015</td>
</tr>
</tbody>
</table>
Trypanosoma_brucei 2 EAN76875 EAN76876

ADFS
Homo_sapiens 3 ENSG00000172757 ENSG00000165410 ENSG00000125868
Gallus_gallus 3 ENSEMBL00000000793 ENSEMBL0000010027 ENSEMBL0000008667
Xenopus_tropicalis 3 ENSEXETG00000012301 ENSEXETG00000003016 ENSEXETG00000026161
Danio_rerio 3 ENSDARG000000021124 ENSDARG00000014106 ENSDARG00000012972
Ciona_intestinalis 1 ENSCING000000023268
Drosophila_melanogaster 1 FBgn0032321
Caenorhabditis_elegans 1 WBGene00007645
Arabidopsis_thaliana 12 AT1G01750 AT5G52360 AT3G46010 AT3G46000 AT5G59890 AT5G59880 AT4G00680 AT3G45990 AT2G31200 AT2G16700 AT4G25590 AT4G34970
Musa_acuminata 27 GSMUA_AchrUn_randomG06230_001 GSMUA_Achr5G24750_001 GSMUA_Achr5G18890_001 GSMUA_Achr1G09160_001 GSMUA_Achr2G16222_001 GSMUA_Achr2G18590_001 GSMUA_Achr1G00290_001 GSMUA_Achr7G0900_001 GSMUA_Achr10G03670_001 GSMUA_Achr6G15320_001 GSMUA_Achr4G29400_001 GSMUA_Achr11G22530_001 GSMUA_Achr2G11510_001 GSMUA_Achr5G12270_001 GSMUA_Achr6G33310_001 GSMUA_Achr7G16650_001 GSMUA_Achr6G15330_001 GSMUA_Achr2G13120_001 GSMUA_Achr8G11370_001 GSMUA_Achr2G17320_001 GSMUA_Achr10G13570_001 GSMUA_Achr11G04040_001 GSMUA_Achr10G02570_001 GSMUA_Achr11G01120_001 GSMUA_Achr9G04050_001 GSMUA_AchrUn_randomG19840_001 GSMUA_Achr11G17570_001 Glycine_max 19 GLYMA11G02670 GLYMA05G37940 GLYMA20G29810 GLYMA11G11390 GLYMA10G32400 GLYMA10G38000 GLYMA15G13141 GLYMA12G03560 GLYMA20G35190 GLYMA09G02241 GLYMA10G05060 GLYMA08G01660 GLYMA01G42780 GLYMA06G0640 GLYMA03G31960 GLYMA13G19430 GLYMA19G34720 GLYMA06G03640 Brassica_rapa 17 Bra013901 Bra011575 Bra006686 Bra006687 Bra012868 Bra037352 Bra028304 Bra029119 Bra017683 Bra020277 Bra020276 Chlamydomonas_reinhardtii 1 CHLREDRAFT_183437 Saccharomyces_cerevisiae 1 YLL050C Aspergillus_nidulans 1 CADANIAG00009011 Neurospora_crassa 1 EFCR00000001811 Schizosaccharomyces_pombe 1 SPAC20G4.06c Entamoeba_histolytica 1 EHL_197480 Phaeodactylum_tricornutum 1 Phatr15613 Trypanosoma_brueci 1 Tb927.3.5180 Dictyostelium_discoideum 8 DDB_G0291970 DDB_G0283367 DDB_G0277833 DDB_G0274057 DDB_G0274589 DDB_G0274059 DDB_G0272568 DDB_G0272646

FORMINS
Homo_sapiens 13 ENSG00000248905 ENSG00000147202 ENSG00000184922 ENSG00000203485 ENSG00000100592 ENSG00000131504 ENSG00000146122 ENSG00000215045 ENSG00000137460 ENSG00000157827 ENSG00000155816 ENSG000000209734 ENSG00000016791 Gallus_gallus 11 ENSEMBL000000009723 ENSEMBL00000012017 ENSEMBL00000005917 ENSEMBL000000010055 ENSEMBL00000006839 ENSEMBL000000016937 ENSEMBL000000010107 ENSEMBL00000012525 ENSEMBL00000010775
ENSGALG00000000694 ENSGALG00000011608
Xenopus_tropicalis 12 ENSXETG000000018104 ENSXETG00000003221
ENSXETG0000018144 ENSXETG0000000581 ENSXETG00000017030
ENSXETG00000016228 ENSXETG00000016085 ENSXETG00000008613
ENSXETG00000018132 ENSXETG00000001839 ENSXETG00000021703
ENSXETG00000002715
Danio_rerio 26 ENSDARG0000000088133 ENSDARG000000091673 ENSDARG00000061778
ENSDARG000000023318 ENSDARG000000090785 ENSDARG00000055713
ENSDARG00000009689 ENSDARG00000095603 ENSDARG00000088538
ENSDARG00000012586 ENSDARG00000009562 ENSDARG00000043472
ENSDARG000000074812 ENSDARG000000078231 ENSDARG00000015102
ENSDARG000000075041 ENSDARG000000075519 ENSDARG00000015059
ENSDARG00000086261 ENSDARG00000011975 ENSDARG00000086384
ENSDARG00000090527 ENSDARG00000028393 ENSDARG00000076103
ENSDARG00000069129 ENSDARG00000076060
Ciona_intestinalis 8 ENSCING00000000000003656 ENSCING0000000018508 ENSCING0000000000006310
ENSCING0000000022867 ENSCING0000000000009569 ENSCING0000000022004 ENSCING00000000000020464
ENSCING00000018508
Drosophila_melanogaster 5 FBgn0000256 FBgn00011202 FBgn0053556 FBgn00025641 FBgn0052138
Caenorhabditis_elegans 4 WBGene00021698 WBGene00000872 WBGene00019030 WBGene00018976
Arabidopsis_thaliana 21 AT3G25500 AT3G05470 AT5G07650 AT2G25050 AT5G07780
AT5G07760 AT1G70140 AT1G31810 AT1G24150 AT4G15200 AT1G42980 AT1G59910
AT2G43800 AT5G07740 AT5G67470 AT5G07770 AT5G54650 AT5G48360 AT3G07540
AT5G58160 AT3G32400
Musa_acuminata 28 GSMUA_Achr11G19290_001 GSMUA_Achr12G5980_001
GSMUA_Achr3G04690_001 GSMUA_Achr2G14090_001 GSMUA_Achr6G18370_001
GSMUA_Achr5G25760_001 GSMUA_Achr4G32680_001 GSMUA_Achr3G23040_001
GSMUA_Achr5G13600_001 GSMUA_Achr1G25970_001 GSMUA_Achr2G11400_001
GSMUA_Achr6G04430_001 GSMUA_Achr3G13420_001 GSMUA_Achr10G04220_001
GSMUA_Achr4G07090_001 GSMUA_Achr9G02450_001 GSMUA_Achr7G23740_001
GSMUA_Achr1G25960_001 GSMUA_Achr8G22610_001 GSMUA_Achr8G07370_001
GSMUA_Achr7G18740_001 GSMUA_Achr7G26410_001 GSMUA_Achr5G13590_001
GSMUA_Achr3G30260_001 GSMUA_Achr3G13430_001 GSMUA_Achr7G26240_001
GSMUA_Achr6G16780_001 GSMUA_Achr7G26420_001
Glycine_max 37 GLYMA09G38160 GLYMA06G41550 GLYMA06G21190 GLYMA17G08230
GLYMA07G32720 GLYMA16G03050 GLYMA19G42230 GLYMA08G40360
GLYMA05G22410 GLYMA17G17460 GLYMA06G45720 GLYMA15G20441
GLYMA03G39620 GLYMA06G19880 GLYMA01G40080 GLYMA17G33930
GLYMA17G11100 GLYMA20G37980 GLYMA12G34350 GLYMA04G34810
GLYMA18G17290 GLYMA02G36446 GLYMA02G36446 GLYMA04G14770
GLYMA01G04430 GLYMA14G11883 GLYMA12G11110 GLYMA12G16606
GLYMA02G15760 GLYMA09G34830 GLYMA10G29300 GLYMA18G48210
GLYMA13G36200 GLYMA07G06440 GLYMA05G08200 GLYMA11G05220
GLYMA04G32990
Brassica_rapa 29 Bra013217 Bra024447 Bra002668 Bra020385 Bra038438 Bra001256
Bra012246 Bra028684 Bra03791 Bra004786 Bra020386 Bra029012 Bra001148 Bra016233
Bra02965 Bra024640 Bra002969 Bra035415 Bra005952 Bra023204 Bra01388 Bra009306
Bra009307 Bra000328 Bra007822 Bra039561 Bra037087 Bra017889 Bra039436
Chlamydomonas_reinhardtii 1 EDP06208
Saccharomyces_cerevisiae 2 YNL271C YIL159W
Neurospora_crassa 1 EFNCRG00000007600
Aspergillus_nidulans 1 CADANIAG000007295
Schizosaccharomyces_pombe 3 SPAC1F5.04c SPCC895.05 SPAC20G4.02c
Entamoeba_histolytica 5 EHI_118260 EHI_125300 EHI_190990 EHI_192460 EHI_197550
Phaeodactylum_tricornutum 1 Phatr46058 Phatr54518 Phatr46029 Phatr54510 Phatr54229
Trypanosoma_brucei 2 Tb927.5.2300 Tb11.02.3470
Dictyostelium_discoideum 10 DDB_G0279607 DDB_G0285589 DDB_G0282245
DDB_G0287295 DDB_G0269626 DDB_G0284519 DDB_G028297 DDB_G0277175
DDB_G0291378 DDB_G0289763

PROFILINS
Homo_sapiens 3 ENSG00000180644 ENSG00000070087 ENSG00000196570
Gallus_gallus 2 ENSGALG0000010410 ENSGALG0000009457
Xenopus_tropicalis 2 ENSXETG0000002090 ENSXETG0000015309
Danio rerio 3 ENSDARG00000012682 ENSDARG0000003952 ENSDARG00000088091
Ciona_Intestinalis 1 NSCINT00000030820
Drosophila_melanogaster 1 FBtr0079233
Caenorhabditis_elegans 13 WBGene00003989 WBGene00003990 WBGene00003991
Arabidopsis_thaliana 5 AT2G19760 AT4G29350 AT5G56600 AT4G29340 AT2G19770
Musa_acuminata 13 GSMUA_Achr10G13990_001 GSMUA_Achr3G17580_001
GSMUA_Achr6G14890_001 GSMUA_Achr5G06510_001 GSMUA_Achr8G20180_001
GSMUA_Achr7G13660_001 GSMUA_Achr2G19390_001 GSMUA_Achr5G09300_001
GSMUA_Achr4G01030_001 GSMUA_Achr1G11580_001 GSMUA_Achr6G22530_001
GSMUA_Achr9G00090_001 GSMUA_Achr1G04950_001
Glycine_max 8 GLYMA08G03650 GLYMA08G03660 GLYMA05G35970 GLYMA01G0920
GLYMA07G15090 GLYMA05G35980 GLYMA08G03640 GLYMA15G02380
Brassica_rapa 13 Bra031097 Bra036707 Bra039018 Bra011099 Bra002804 Bra006865
Bra014545 Bra010331 Bra024148 Bra039019 Bra031101 Bra011098 Bra036705
Chlamydomonas_reinhardtii 1 CHLREDAF 1811887
Saccharomyces_cerevisiae 1 YOR122C
Neurospora_crassa 1 EFNCRG00000006087
Aspergillus_nidulans 1 CADANIAG000009205
Schizosaccharomyces_pombe 1 SPAC4A8.15c
Entamoeba_histolytica 1 EHI_176140
Phaeodactylum_tricornutum 0
Trypanosoma_brucei 1 Tb11.01.3530
Dictyostelium_discoideum 3 DDB_G0287125 DDB_G0286187 DDB_G0271142

TROPOMODULINS/LEIOMODINS
Homo_sapiens 7 ENSG00000136842 ENSG00000128872 ENSG00000163157
ENSG0000017807 ENSG00000163380 ENSG00000138594 ENSG00000163431
Gallus_gallus 7 ENSGALG0000002125 ENSGALG0000004710 ENSGALG0000011948
ENSGALG0000008805 ENSGALG00000018946 ENSGALG00000028721
ENSGALG0000004740
Xenopus_tropicalis 7 ENSXETG00000010173 ENSXETG00000020749 ENSXETG0000008139
ENSXETG00000020747 ENSXETG0000016168 ENSXETG0000007976
ENSXETG0000022132
Danio rerio 8 ENSDARG00000078318 ENSDARG0000002571 ENSDARG0000045864
ENSDARG00000062662 ENSDARG00000077261 ENSDARG0000056111
ENSDARG00000020890 ENSDARG00000045634
<table>
<thead>
<tr>
<th>Species</th>
<th>Gene (symbol)</th>
<th>Chromosome</th>
<th>Accession Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo sapiens 20</td>
<td>TPM1</td>
<td>Chromosome 15</td>
<td>NM_000366.5 NM_001018004.1 NM_001018005.1 NM_001018006.1 NM_001018007.1 NM_001018008.1 NM_001018020.1; TPM2 Chromosome 9 NM_003289.3 NM_213674.1; TPM3 Chromosome 1 NM_001043351.1 NM_001043352.1 NM_001043353.1 NM_001278188.1 NM_001278189.1 NM_001278190.1 NM_001278191.1 NM_152263.3 NM_153649.3; TPM4 Chromosome 19 NM_001145160.1 NM_003290.2</td>
</tr>
<tr>
<td>Gallus gallus 12</td>
<td>TPM1</td>
<td>Chromosome 10</td>
<td>NM_205401.1 M32441.1 M36337.1 P04268-2 P04268-6 P04268-7; TPM2 Chromosome Z NM_205446.1 M23081.1 M23082.1 M64288.1; TPM3 Chromosome 25 NM_001245927.1</td>
</tr>
<tr>
<td>Xenopus tropicalis 7</td>
<td>TPM1</td>
<td>Chromosome 25</td>
<td>NM_001050528.1 BC158431.1 BC168590.1; TPM2 NM_001030416.1; TPM3 NM_203968.1; TPM4 NM_001278191.1 NM_001278190.1 NM_001278189.1 NM_001278188.1 NM_001278187.1 NM_001278186.1 NM_001278185.1 NM_001278184.1 NM_001278183.1 NM_001278182.1</td>
</tr>
<tr>
<td>Danio rerio 48</td>
<td>TPM1</td>
<td>Chromosome 25</td>
<td>NM_001102629.2 NM_001293168.1 NM_200934.3 XM_005174370.1 XM_005174376.1 XM_005174372.1 XM_005174378.1 XM_005174380.1 XM_005174383.1 XM_005174371.1 XM_005174377.1 XM_005174384.1 XM_005174385.1 XM_005174373.1 XM_005174381.1 XM_005174374.1 XM_005174379.1 XM_005174375.1</td>
</tr>
<tr>
<td>Ciona intestinalis 15</td>
<td>CeTM (LEV-11)</td>
<td>Chromosome 1</td>
<td>Y105E8B.1d Y105E8B.1g Y105E8B.1a Y105E8B.1b Y105E8B.1c XM_002119087.1; CTM1 Chromosome 14 NM_001032538.1</td>
</tr>
<tr>
<td>Drosophila melanogaster 22</td>
<td>Tm1</td>
<td>Chromosome 3R</td>
<td>NM_169635.3 NM_206495.2 NM_169637.4 NM_001260190.1 NM_169633.5 NM_169638.4 NM_001275662.1 NM_001170148.2 NM_169634.3 NM_169636.3 NM_206494.3 NM_169639.3 NM_169640.4 NM_169641.4 NM_079643.4 NM_001275661.1 NM_001260191.1; Tm2 Chromosome 3R NM_079637.5 NM_169645.4 NM_001275664.1 NM_169644.2 NM_001275663.1</td>
</tr>
<tr>
<td>Caenorhabditis elegans 1</td>
<td>CeTm-like Protein</td>
<td>Chromosome 3</td>
<td>XM_002119087.1; CTM1 Chromosome 14 NM_001032538.1</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae 2</td>
<td>Tm1</td>
<td>Chromosome 14</td>
<td>YNL079C; Tm2 Chromosome 9 YIL138C</td>
</tr>
<tr>
<td>Neurospora crassa 1</td>
<td>Tm1</td>
<td>ENSGALG00000013056 ENSGALG00000011324 ENSGALG00000013045 ENSGALG00000010439 ENSGALG0000008444 ENSGALG00000015017</td>
<td></td>
</tr>
<tr>
<td>Aspergillus nidulans 1</td>
<td>TpmA</td>
<td>Chromosome 5</td>
<td>CADANIAT00003379</td>
</tr>
<tr>
<td>Schizosaccharomyces pombe 1</td>
<td>Cdc8</td>
<td>Chromosome 1</td>
<td>SPAC27F1.02c.1</td>
</tr>
</tbody>
</table>

**TUBULIN**

<table>
<thead>
<tr>
<th>Species</th>
<th>Gene (symbol)</th>
<th>Accession Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo sapiens 24</td>
<td>ENSG000000183785 ENSG00000075886 ENSG000000152086 ENSG000000167552 ENSG000000167553 ENSG000000198033 ENSG000000123416 ENSG000000127824 ENSG000000074935 ENSG000000188229 ENSG000000137267 ENSG000000196230 ENSG000000173876 ENSG000000261812 ENSG000000173213 ENSG000000258947 ENSG000000198211 ENSG000000176014 ENSG000000137285 ENSG000000101162 ENSG000000104833 ENSG000000131462 ENSG000000108423 ENSG00000037042</td>
<td></td>
</tr>
<tr>
<td>Gallus gallus 17</td>
<td>ENSGALG000000013056 ENSGALG00000006241 ENSGALG0000000433 ENSGALG000000011324 ENSGALG00000013045 ENSGALG00000010439 ENSGALG00000008444 ENSGALG00000015017 ENSGALG0000000059 ENSGALG00000027684 ENSGALG00000012821 ENSGALG00000029076 ENSGALG00000026802 ENSGALG0000008586 ENSGALG0000007447 ENSGALG00000003150 ENSGALG0000005173</td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>Accession Numbers</td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td><em>Brassica rapa</em></td>
<td>Bra007622, Bra015815, Bra018825, Bra020061, Bra020062, Bra006517, Bra039648, Bra010114, Bra002260, Bra039987, Bra002261, Bra020572, Bra033737, Bra038795, Bra018379, Bra020912, Bra033796, Bra005395, Bra039506, Bra041172, Bra018184, Bra003116, Bra012923, Bra014232, Bra013481, Bra019493, Bra009710, Bra008903, Bra027544</td>
<td></td>
</tr>
<tr>
<td><em>Chlamydomonas reinhardtii</em></td>
<td>CHLREDRAFT_188933, CHLREDRAFT_186023, CHLREDRAFT_188195, CHLREDRAFT_129868, CHLREDRAFT_128523, CHLREDRAFT_136082, CHLREDRAFT_129876</td>
<td></td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>YML085C, YML124C, YFL037W, YLR212C</td>
<td></td>
</tr>
<tr>
<td><em>Neurospora crassa</em></td>
<td>EFNCRG00000009281, EFNCRG00000006435, EFNCRG00000003612</td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus nidulans</em></td>
<td>CADANIAG00002396, CADANIAG00001997, CADANIAG00000677, CADANIAG00005302, CADANIAG00005306, CADANIAG00007636, CADANIAG00001443</td>
<td></td>
</tr>
<tr>
<td><em>Schizosaccharomyces pombe</em></td>
<td>SPBC16A3.15c, SPBC800.05c, SPBC26H8.07c, SPBC32F12.04</td>
<td></td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td>EHI_005950, EHI_010530, EHI_049920, EHI_167010, EHI_008240</td>
<td></td>
</tr>
<tr>
<td><em>Phaeodactylum tricornutum</em></td>
<td>Phatr54534, Phatr44225, Phatr21122</td>
<td></td>
</tr>
<tr>
<td><em>Trypanosoma brucei</em></td>
<td>Tb927.1.2400, Tb927.1.2340, Tb927.3.910, Tb927.1.2350, Tb927.1.2330, Tb927.1.2380, Tb927.1.2390, Tb927.1.2370, Tb10.70.6950, Tb11.01.2695, Tb927.1.2360</td>
<td></td>
</tr>
<tr>
<td><em>Dictyostelium discoideum</em></td>
<td>DDB_G0287689, DDB_G0281889, DDB_G0271738, DDB_G0269196</td>
<td></td>
</tr>
</tbody>
</table>