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Formins Determine the Functional Properties of Actin Filaments in Yeast

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Summary

The actin cytoskeleton executes a broad range of essential functions within a living cell. The dynamic nature of the actin polymer is modulated to facilitate specific cellular processes at discrete locations by actin-binding proteins (ABPs), including the formins and tropomyosins (Tms). Formins nucleate actin polymers, while Tms are conserved dimeric proteins that form polymers along the length of actin filaments. Cells possess different Tm isoforms, each capable of differentially regulating the dynamic and functional properties of the actin polymer. However, the mechanism by which a particular Tm localizes to a specific actin polymer is unknown. Here we show that specific formin family members dictate which Tm isoform will associate with a particular actin filament to modulate its dynamic and functional properties at specific cellular locations.

Exchanging the localization of the fission yeast formins For3 and Cdc12 results in an exchange in localizations of Tm forms on actin polymers. This nucleator-driven switch in filament composition is reflected in a switch in actin dynamics, together with a corresponding change in the filament’s ability to regulate ABPs and myosin motor activity. These data establish a role for formins in dictating the filament’s ability to regulate ABPs and myosin motor activity. This illustrated the presence of interphase actin filaments that only detect Tm in its acetylated state [13]. This illustrated the presence of interphase actin filaments that only detect Tm in its acetylated state [13]. This illustrated the presence of interphase actin filaments that only detect Tm in its acetylated state [13]. This illustrated the presence of interphase actin filaments that only detect Tm in its acetylated state [13]. This illustrated the presence of interphase actin filaments that only detect Tm in its acetylated state [13]. This illustrated the presence of interphase actin filaments that only detect Tm in its acetylated state [13]. This illustrated the presence of interphase actin filaments that only detect Tm in its acetylated state [13].

Results and Discussion

The actin cytoskeleton plays a pivotal role in facilitating growth during the life cycles of eukaryotes. Regulated by accessory proteins, actin polymerizes into dynamic filaments, which can bundle to form cables and act as tracks for myosin motors. Actin is nucleated by the Arp2/3 complex and formins, a conserved group of proteins that catalyze the processive movement of actin filaments. Formins nucleate actin polymers, while Tms are conserved dimeric proteins that form polymers along the length of actin filaments. Cells possess different Tm isoforms, each capable of differentially regulating the dynamic and functional properties of the actin polymer. However, the mechanism by which a particular Tm localizes to a specific actin polymer is unknown. Here we show that specific formin family members dictate which Tm isoform will associate with a particular actin filament to modulate its dynamic and functional properties at specific cellular locations.

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The ability of each fusion to nucleate actin was assessed using a GFP actin label (Figure 1B) and immunostaining all fission yeast TmCdc8 using an anti-TmCdc8 antibody (Figure S1) [11]. This illustrated the presence of interphase actin filaments in for3J cdc12-112 cells expressing Tea1-ForminF, Tea1-ForminCdc12, or the for3J gene. In stark contrast antibodies that only detect Tm in its acetylated state [13] gave no signal in the for3J Tea1-ForminF cells and staining of interphase actin filaments in the Tea1-ForminCdc12 cells (Figures 1C and S1). In addition strongly stained arrays of actin-TmCdc8 cables were often observed in interphase cells expressing Tea1-Cdc12, signifying the composition of the actin bundles is different from normal ForminF- and -Cdc12-nucleated interphase actin polymers (Figures 1B and S1). Thus, acetylated Tm only associates with Cdc12-nucleated filaments, indicating that the formin at the tip of the actin filament either directly or indirectly specifies which Tm is recruited onto the actin polymer.

The impact that each formin-Tm combination had upon the dynamic nature of actin polymers was assessed using either LifeAct or a calponin homology domain GFP fusion (CHD Rng2) [18, 19] (Figures 2A and S2; Table S1; Movie S1). Growth rates of for3J cells expressing ForminF and GFP-CHD Rng2 did not differ from wild-type when expression from the nmt41 gene. In stark contrast antibodies that only detect Tm in its acetylated state [13] gave no signal in the for3J Tea1-ForminF cells and staining of interphase actin filaments in the Tea1-ForminCdc12 cells (Figures 1C and S1). In addition strongly stained arrays of actin-TmCdc8 cables were often observed in interphase cells expressing Tea1-Cdc12, signifying the composition of the actin bundles is different from normal ForminF- and -Cdc12-nucleated interphase actin polymers (Figures 1B and S1). Thus, acetylated Tm only associates with Cdc12-nucleated filaments, indicating that the formin at the tip of the actin filament either directly or indirectly specifies which Tm is recruited onto the actin polymer.

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the formin and Tm affect the recruitment of the CHD\textsuperscript{Rng2} to actin polymers.

These findings indicate that formins specify which Tm associates with actin to provide a mechanism for modulating the conformation and dynamics of the polymer at discrete cellular locations. The ability of each formin-nucleated actin-Tm Cdc8 polymer to impact the distribution and activity of ABPs was further tested in the following three ways.

First, we determined whether the different form-actin-Tm\textsuperscript{Cdc8} polymers affected the localization of cofilin (Adf1/Cof1) and the fission yeast IQGAP (Rng2). We tested whether the formin and Tm affect cofilin distribution as specific Tm isoforms can recruit cofilin to the actin polymer in mammalian cells [3]. However, simultaneous observation of GFP-Adf1 localization in wild-type cells and for3\textsuperscript{+} cells expressing Tea1-For3 revealed that modulating the Tm\textsuperscript{Cdc8} composition of S. pombe actin cables did not affect the distribution of cofilin (Figure 2B). In contrast, while IQGAP\textsuperscript{Rng2} normally localizes to the CAR during mitosis, expressing Tea1-Cdc12 caused the protein to localize to foci at the end of for3\textsuperscript{+} cells (Figures 2C and 2D), illustrating that formins play a role in dictating the cellular localization of the IQGAP.

Second, we examined the impact of each actin-Tm\textsuperscript{Cdc8} complex on the localization and movement of the yeast myosin V, Myo52. While Myo52-mCherry signal was dispersed throughout the cytoplasm in control for3\textsuperscript{+} cells, Myo52 foci moved along actin filaments to concentrate at sites of cell growth in for3\textsuperscript{+} cells expressing either Tea1-For3 or Tea1-Cdc12 formin fusions (Figure 2E), demonstrating that interphase actin polymers nucleated from each formin fusion can propagate myosin movement. Myo52 moved along Tea1-Cdc12-nucleated interphase actin filaments at 75% of the velocity of filaments nucleated by Tea1-For3 (Table 1) or in wild-type cells [21] (different at a level of confidence of 99%). While the formin has an impact upon the cellular movement of Myo52 within the cell, this may be a consequence of the change in actin polymerization rate. However the reduction in actin-based Myo52 motility was reflected in the slower growth rate of Tea1-Cdc12 cells (Table 1).
Figure 2. Tea1-Cdc12 and Tea1-For3 Nucleate Functional Actin Filaments that Differentially Affect Interactions with ABPs

(A) Maximum projections from subsecond time-lapse z stacks illustrate actin polymer dynamics in for3Δ tea1-for3-mCherry cells.
(B) YFP-cofilin (green) association with cortical actin patches is equivalent in wild-type (no Cherry signal, marked with asterisks) and for3Δ cells expressing Tea1-Cdc12 (magenta).
(C and D) YFP-Rng2 localizes to the cell poles in for3Δ tea1-for3-mCherry cells (C) and exclusively to the CAR in wild-type cells (D).
(E) Maximum projections of mCherry-labeled (magenta) Myo52 in for3Δ cells expressing Tea1 (left), Tea1-For3 (middle), or Tea1-Cdc12 (right) GFP carboxyl fusion proteins (green). Myo52 moved along actin polymers in for3Δ cells expressing either Tea1-formin fusion, but not in cells expressing Tea1-GFP alone. Scale bars, 5 μm.
Third, the ability of each formin to nucleate actin polymers at the CAR was assessed. The carboxyl-terminal half of the myosin II (Myo2) coiled-coil tail alone localizes to the cell equator and CAR in early mitosis [22]. Fusions between the C-terminal half of the Myo2 tail (Myo2T) and either Formin For3 or Formin Cdc12 were generated (Myo2T-For3 and Myo2T-Cdc12; Figure S3), and their ability to localize to the CAR and complement Formin Cdc12 function was monitored in cdc12-112 cells at 36°C. In contrast to cdc12Δ cells that lack any medial actin filaments, mitotic cdc12-112 cells contain randomly organized medial wisps of actin [15] and Myo2 (Figure S3) at the restrictive temperature. Similarly Myo2 incorporated into disorganized medial filaments in cells expressing Tea1-For3, Tea1-Cdc12, or Myo2T (Figure 3A). Intriguingly, Myo2 associates with filament-like structures emanating from the tip of interphase cdc12-112 tea1-cdc12 cells, indicating the Cdc12 formin can affect the distribution of Myo2 within interphase cells. In contrast, Myo2T-Cdc12 and Myo2T-For3 incorporated into a CAR, which contracted with kinetics similar to wild-type (Figure 3B). However, the CAR collapsed in the majority of cdc12-112 cells expressing Myo2T-For3 upon constriction (Figure 3A,v), and cells stopped dividing within two divisions (Figure S3). Immunofluorescence

<table>
<thead>
<tr>
<th>Strain</th>
<th>Wild-Type</th>
<th>for3Δ</th>
<th>for3Δ tea1-cdc12</th>
<th>cdc12-112</th>
<th>cdc12-112 myo2T-for3</th>
<th>cdc12-112 myo2T-cdc12</th>
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<tbody>
<tr>
<td>% interphase cells with bipolar formin localization</td>
<td>ND</td>
<td>45%</td>
<td>71%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
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<tr>
<td>Growth rate of polar actin filaments (µm/s)</td>
<td>0.57 ± 0.09</td>
<td>0.59 ± 0.10</td>
<td>0.79 ± 0.13</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Shrinkage rate of polar actin filaments (µm/s)</td>
<td>0.57 ± 0.10</td>
<td>0.79 ± 0.17</td>
<td>0.83 ± 0.14</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>GFP-CHD^pro2 intensity on polar actin filaments (a.u./µm²)</td>
<td>ND</td>
<td>141</td>
<td>107</td>
<td>100%</td>
<td>98%</td>
<td>99%</td>
</tr>
<tr>
<td>Mean velocity of Myo52 (µm/s)</td>
<td>0.58 ± 0.05</td>
<td>0.59 ± 0.05</td>
<td>0.44 ± 0.01</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>% mitotic cells with formin localized to contractile CAR</td>
<td>0%</td>
<td>0%</td>
<td>32%</td>
<td>100%</td>
<td>98%</td>
<td>99%</td>
</tr>
<tr>
<td>Complement cdc12-112</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>% interphase cells with aceT^mCdc8-actin cables*</td>
<td>0%</td>
<td>0%</td>
<td>83%</td>
<td>0%</td>
<td>0%</td>
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<tr>
<td>% mitotic cells with aceT^mCdc8 CAR</td>
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<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>72%</td>
<td>0%</td>
</tr>
</tbody>
</table>

*Defined as an interphase cell containing at least one anti-aceT^mCdc8 antibody-labeled cable (see Figure S3). n > 200.

Growth rate, shrinkage rate, and mean velocity were determined using methods described in Supplemental Experimental Procedures. ND, not determined.

Table 1. Properties of Cells Expressing Formins and Formin Fusions

Figure 3. A Myo2T-For3 Fusion Recruits to the Cell Equator and Nucleates Partially Functional Contractile Rings Containing Unacetylated Cdc8

(A) mCherry (magenta) and GFP (green) signal in cdc12-112 myo2-mCherry cells expressing Myo2T (i), Tea1-For3 (ii), Tea1-Cdc12 (iii), Myo2T-Cdc12 (iv), or Tea1-Cdc12 (v) GFP fusions, cultured at 36°C for 4 hr. While Myo2 associated with Tea1-Cdc12-nucleated interphase actin filaments (iii, arrow), only cells expressing the Myo2T-Cdc12 (iv) or Myo2T-For3 (v) fusions formed a CAR.

(B) Myo2 rings were seen to constrict within 40 min of forming in all myo2-mCherry cells (i) and 5% of myo2-mCherry cdc12-112 cells expressing Myo2T-For3 (ii) when cultured at 36°C.

(C) Immunofluorescence of wild-type (left) and cdc12-112 (right) cells expressing Myo2T-For3 using anti-Cdc8^unace antibodies revealed that aceT^mCdc8 decorated the medial ring in cells expressing the Myo2T-For3 fusion.

Scale bars, 5 µm.
revealed that unace Tm<sup>Cdc8</sup>, which associates exclusively with interphase actin polymers and not to the CAR in wild-type cells [13], localized to the CAR in the presence of Myo2T-For3 (Figure 3C; Movie S2), supporting the hypothesis that the formin determines which Tm form associates with the growing actin polymer. These data are consistent with cells lacking acetylated Tm<sup>Cdc8</sup> frequently forming unstable CARs [13], as only acetylated Tm<sup>Cdc8</sup> can form the stable polymers that are capable of stabilizing actin and regulating Myo2 appropriately [13, 23]. Intriguingly, as for Formin<sup>Cdc12</sup>, For3-dependent actin nucleation is not restricted by location, suggesting that either formins are constitutively active, their accumulation at a discrete cellular location is sufficient to promote actin polymerization activity, or autoinhibition of Cdc12 activity is abolished by amino-terminal fusions. These data demonstrate that formins (1) define the functional properties of the actin filaments they nucleate in vivo, (2) regulate the Tm isoform that is recruited to the actin polymer and modulate the affinities of other ABPs for the actin polymer, and (3) can nucleate functional actin polymers independent of normal cell-cycle-dependent spatial and temporal constraints. Cells can express multiple forms of Tm (>40 isoforms in humans), each localizing to a discrete cellular location where they differentially modulate the physical properties of actin polymers to facilitate specific functions [9]. Acetylation offers a level of control, additional to variation in sequence, to modify the physical properties of Tms as well as providing a signal to facilitate their recruitment to different actin structures. Formins may modulate the recruitment of associating proteins directly or by modifying conformations in the actin polymer. Formins are not only located at the barbed end of actin filaments, where they interact with the amino termini of the Tm filament incorporating onto the growing actin polymer [24], but also associate with lateral surfaces of the actin filament [25, 26]. Tm can enhance or block ABP binding to actin [9], but formin may also modulate the pitch of the actin filament directly to change affinities for multiple ABPs [24, 26, 27]. A picture is emerging wherein formins orchestrate a delicate Tm-dependent interplay between ABPs to modulate the functional properties of the actin polymer, allowing actin to facilitate diverse dynamic processes within the cell.

Supplemental Information

Supplemental Information includes three figures, three tables, Supple-
mental Experimental Procedures, and two movies and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.05.034.

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