Current Biology 24, 1525–1530, July 7, 2014 ©2014 Elsevier Ltd All rights reserved http://dx.doi.org/10.1016/j.cub.2014.05.034

Report

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 which specific Tm variant will associate with a growing actin filament and therefore specify the functional capacity of the actin filaments that they create.

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 addition of actin monomers to the barbed end of the polymer [1]. The dynamic and functional properties of different actin polymers are temporally and spatially regulated to facilitate discrete functions through association with actin-binding proteins (ABPs), including tropomyosins (Tms). Tms persist in multiple forms within the cell, each associating with actin at distinct locations to modify the nature of the actin polymers [2–4] to facilitate specific cellular functions [5–9].

Schizosaccharomyces pombe possesses a single Tm, Cdc8 [10], that persists in both amino-terminally acetylated ($_{ace}Tm^{Cdc8}$) and unacetylated ($_{unace}Tm^{Cdc8}$) forms [11]. Acetylation stabilizes Tm^{Cdc8} polymers to promote tight actin binding and regulate myosins. In contrast, $_{unace}Tm^{Cdc8}$ filaments form a weaker, more flexible interaction with actin and are unable to regulate actomyosin interactions to the same extent as $_{ace}Tm^{Cdc8}$ [11, 12]. Each form localizes to distinct actin structures within the yeast cell: $_{ace}Tm^{Cdc8}$ localizes to the cytokinetic actomyosin ring (CAR) during mitosis, while $_{unace}Tm^{Cdc8}$ localizes to dynamic cytoplasmic actin polymers that extend from the cell tips during interphase [13]. Each actin structure has distinct dynamic properties and is nucleated by different formins: Cdc12 ($Formin^{Cdc12}$) at the CAR during early mitosis, and For3 ($Formin^{For3}$) at the growing ends of the cell during interphase.

We asked whether formins regulate actin function by determining the ABP (including Tm) composition of different actin structures. To this end, we generated constructs encoding formin-fusion proteins that exchanged the normal distribution of $Formin^{For3}$ and $Formin^{Cdc12}$ [14, 15]. Tea1 is a polarity factor delivered on microtubules to the cell poles, where it is anchored to the membrane by Tea4/Wsh3 [16, 17]. Fusion of Tea1 to the amino terminus of either $Formin^{Cdc12}$ or $Formin^{For3}$ (generating Tea1-$Formin^{Cdc12}$ and Tea1-$Formin^{For3}$) targeted both formins to the cell poles of wild-type, *for3*, *tea1*, and *tea4* deletion cells (Figure 1A; see also Figure S1 available online).

The ability of each fusion to nucleate actin was assessed using a GFP actin label (Figure 1B) and immunostaining all fission yeast Tm^{Cdc8} using an anti-Tm^{Cdc8} antibody (Figure S1) [11]. This illustrated the presence of interphase actin cables in *for3Δ cdc12-112* cells expressing Tea1-$Formin^{For3}$, Tea1-$Formin^{Cdc12}$, or the *for3*$^{+}$ gene. In stark contrast antibodies that only detect Tm in its acetylated state [13] gave no signal in the *for3Δ* Tea1-$Formin^{For3}$ cells and staining of interphase actin filaments in the Tea1-$Formin^{Cdc12}$ cells (Figures 1C and S1). In addition strongly stained arrays of actin-Tm^{Cdc8} cables were often observed in interphase cells expressing Tea1-Cdc12, signifying the composition of the actin bundles is different from normal $Formin^{For3}$-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 *for3Δ* cells expressing $Formin^{For3}$ and GFP-CHD^{Rng2} did not differ from wild-type when expression from the *nmt41* promoter was partially suppressed by adding 4 pmol thiamine. Results were consistent between fluorescent actin markers. Actin filaments nucleated by $Formin^{Cdc12}$ from the cell poles grew 34% faster than those nucleated from $Formin^{For3}$ or in wild-type cells (Table 1). This is consistent with in vitro studies indicating that formins differentially modulate the rate of actin polymerization [20]. While filament shrinkage rates were equivalent, mean CHD^{Rng2} fluorescence was 32% higher on polymers nucleated from For3 (Table 1). This observation is consistent with $_{ace}Tm^{Cdc8}$ filaments sitting in a more stable position on the actin polymer and raising the possibility that

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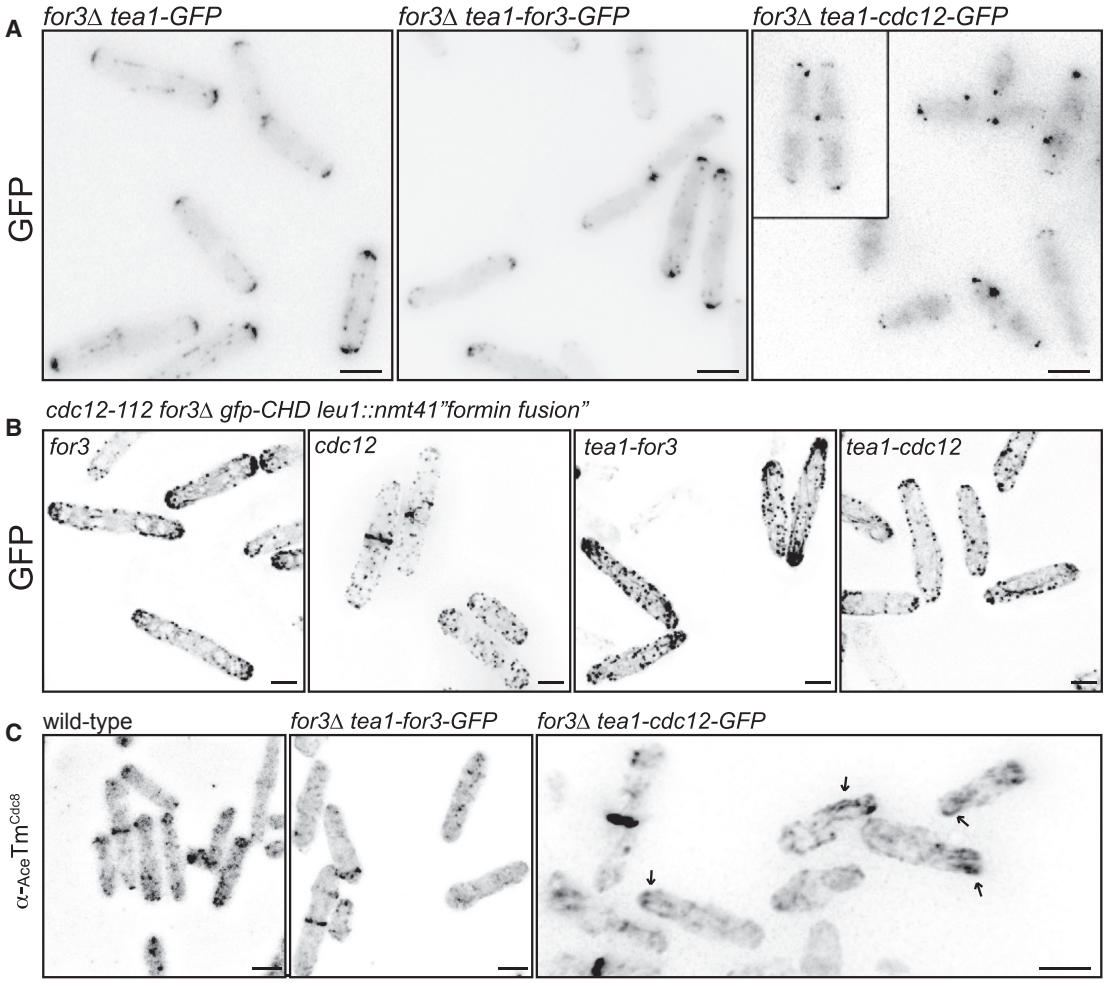

Figure 1. Formins Determine Dynamics and Tm Composition of Actin Polymers

(A) GFP signal concentrated to the cell poles of *for3Δ* cells expressing Tea1-GFP (left), Tea1-For3-GFP (middle), or Tea1-Cdc12-GFP (right) fusion proteins.
(B) GFP-labeled actin structures in *for3Δ cdc12-112 GFP-CHD^{rng2}* cells incubated at 36°C for 4 hr, in which DNA encoding formins or formin fusions had been integrated into the chromosomal *leu1$^+$* locus. Interphase actin cables can be seen only in cells expressing *for3$^+$*, *tea1-for3*, or *tea1-cdc12*.
(C) Anti-acetylated Cdc8 immunofluorescence of wild-type (left), *for3Δ tea1-for3* (middle), or *for3Δ tea1-cdc12* cells revealed that $_{ace}$TM^{Cdc8}-decorated interphase actin filaments (arrows) were only present in cells expressing *tea1-cdc12*.
Scale bars, 5 μm.

the formin and Tm affect the recruitment of the CHD^{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^{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 *for3Δ* cells expressing Tea1-Cdc12 revealed that modulating the Tm^{Cdc8} composition of *S. pombe* actin cables did not affect the distribution of cofilin (Figure 2B). In contrast, while IQGAP^{Rng2} normally localizes to the CAR during mitosis, expressing Tea1-Cdc12 caused the protein to localize to foci at the end of *for3Δ* 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^{Cdc8} complex on the localization and movement of the yeast myosin V, Myo52. While Myo52-mCherry signal was dispersed throughout the cytoplasm in control *for3Δ* cells, Myo52 foci moved along actin filaments to concentrate at sites of cell growth in *for3Δ* 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).

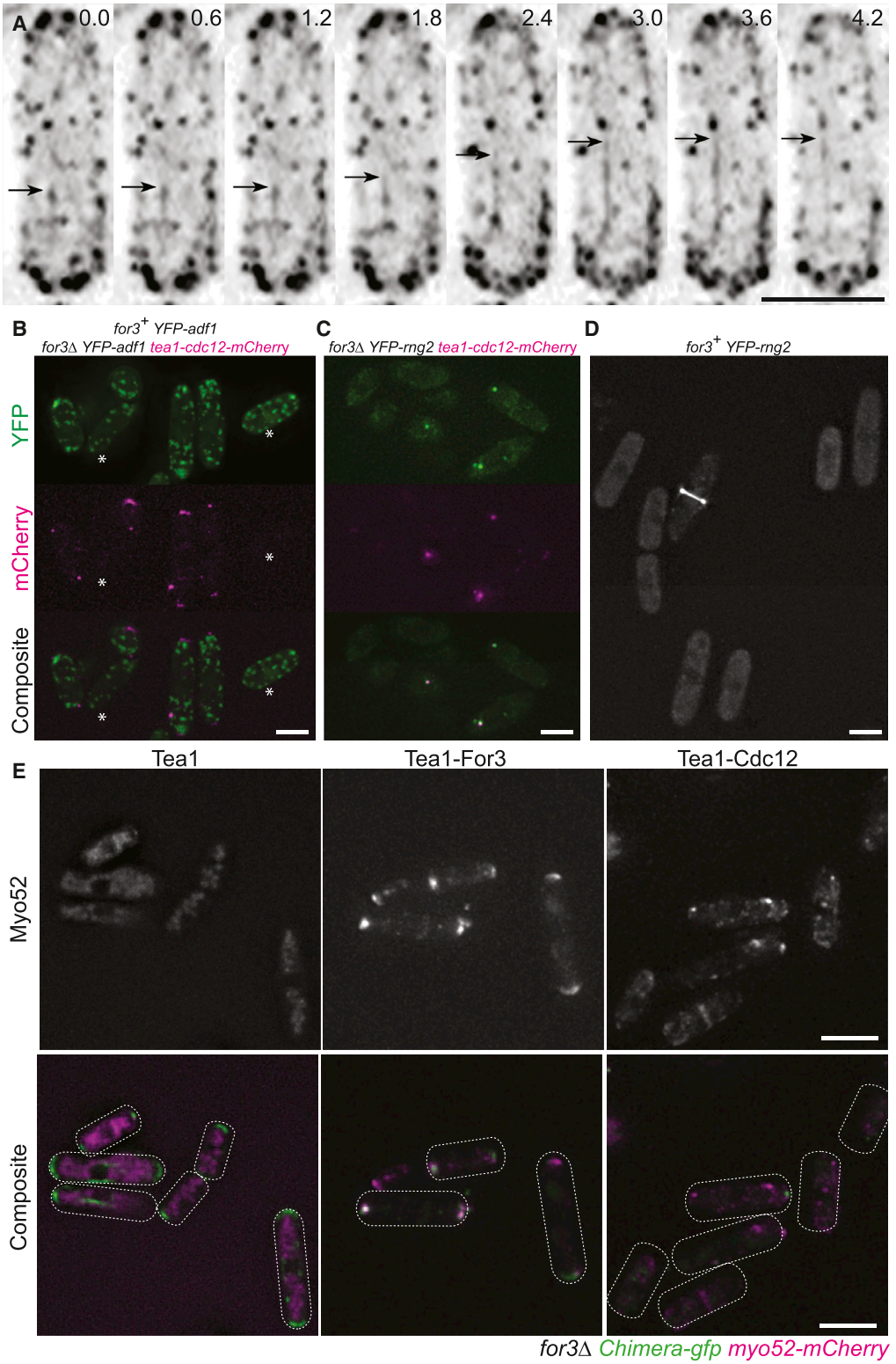

Figure 2. Tea1-Cdc12 and Tea1-For3 Nucleate Functional Actin Filaments that Differentially Affect Interactions with ABPs

(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.

Table 1. Properties of Cells Expressing Formins and Formin Fusions

Strain	Wild-Type	*for3Δ for3*	*for3Δ tea1-cdc12*	*cdc12-112 cdc12*	*cdc12-112 myo2T-for3*	*myo2T-cdc12*
% interphase cells with bipolar formin localization	ND	45%	71%	0%	0%	0%
Growth rate of polar actin filaments (μm/s)	0.57 ± 0.09	0.59 ± 0.10	0.79 ± 0.13	ND	ND	ND
Shrinkage rate of polar actin filaments (μm/s)	0.77 ± 0.10	0.79 ± 0.17	0.83 ± 0.14	ND	ND	ND
GFP-CHD^{Rng2} intensity on polar actin filaments (a.u./μm^2)	ND	141	107	ND	ND	ND
Mean velocity of Myo52 (μm/s)	0.56 ± 0.05	0.59 ± 0.05	0.44 ± 0.01	ND	ND	ND
% mitotic cells with formin localized to contractile CAR	0%	0%	32%	100%	98%	99%
Complement *cdc12-112*	–	–	–	+	–	+
% interphase cells with $_{ace}$Tm^{Cdc8}-actin cables[a]	0%	0%	83%	0%	0%	0%
% mitotic cells with $_{unace}$Tm^{Cdc8} CAR	0%	0%	0%	0%	72%	0%

Growth rate, shrinkage rate, and mean velocity were determined using methods described in Supplemental Experimental Procedures. ND, not determined.
[a]Defined as an interphase cell containing at least one anti-$_{ace}$Tm^{Cdc8} antibody-labeled cable (see Figure S3). n > 200.

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 3Av), and cells stopped dividing within two divisions (Figure S3). Immunofluorescence

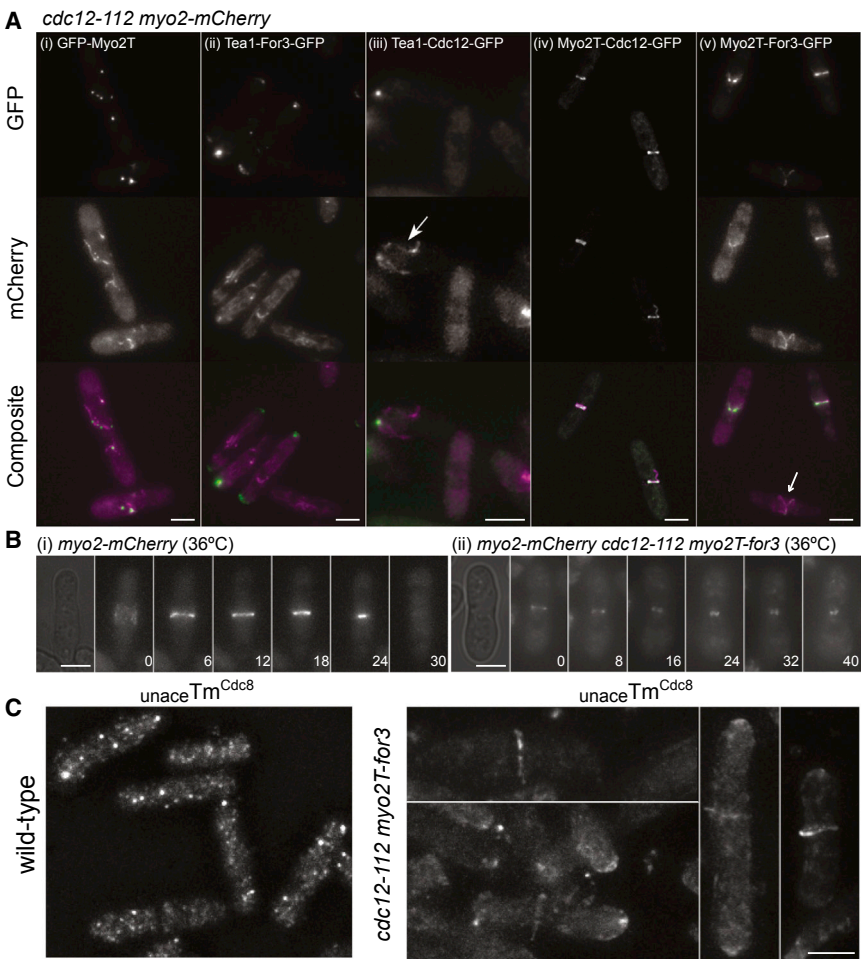

Figure 3. A Myo2T-For3 Fusion Recruits to the Cell Equator and Nucleates Partially Functional Contractile Rings Containing Unacetylated Cdc8

(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 $_{unace}$Tm^{Cdc8} decorated the medial ring in cells expressing the Myo2T-For3 fusion.

Scale bars, 5 μm.

revealed that $_{unace}Tm^{Cdc8}$, 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^{Cdc8} frequently forming unstable CARs [13], as only acetylated Tm^{Cdc8} can form the stable polymers that are capable of stabilizing actin and regulating Myo2 appropriately [13, 23]. Intriguingly, as for $Formin^{Cdc12}$, 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 can 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 [8], 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, Supplemental 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.

Acknowledgments

We thank F. Chang, I. Hagan, and P. Nurse for strains. This work was supported by funding from the Biotechnology and Biological Sciences Research Council (BB/F011784/1 and BB/H016066/1), a CASE industrial bursary from Cairn Research Ltd. to M.J., and a Royal Society Industry Fellowship to D.P.M.

Received: February 3, 2014
Revised: April 25, 2014
Accepted: May 14, 2014
Published: June 19, 2014

References

1. Breitsprecher, D., and Goode, B.L. (2013). Formins at a glance. J. Cell Sci. *126*, 1–7.
2. Creed, S.J., Desouza, M., Bamburg, J.R., Gunning, P., and Stehn, J. (2011). Tropomyosin isoform 3 promotes the formation of filopodia by regulating the recruitment of actin-binding proteins to actin filaments. Exp. Cell Res. *317*, 249–261.
3. Bryce, N.S., Schevzov, G., Ferguson, V., Percival, J.M., Lin, J.J., Matsumura, F., Bamburg, J.R., Jeffrey, P.L., Hardeman, E.C., Gunning, P., and Weinberger, R.P. (2003). Specification of actin filament function and molecular composition by tropomyosin isoforms. Mol. Biol. Cell *14*, 1002–1016.
4. Tojkander, S., Gateva, G., Schevzov, G., Hotulainen, P., Naumanen, P., Martin, C., Gunning, P.W., and Lappalainen, P. (2011). A molecular pathway for myosin II recruitment to stress fibers. Curr. Biol. *21*, 539–550.
5. Ujfalusi, Z., Vig, A., Hild, G., and Nyitrai, M. (2009). Effect of tropomyosin on formin-bound actin filaments. Biophys. J. *96*, 162–168.
6. Skau, C.T., Neidt, E.M., and Kovar, D.R. (2009). Role of tropomyosin in formin-mediated contractile ring assembly in fission yeast. Mol. Biol. Cell *20*, 2160–2173.
7. Ujfalusi, Z., Kovács, M., Nagy, N.T., Barkó, S., Hild, G., Lukács, A., Nyitrai, M., and Bugyi, B. (2012). Myosin and tropomyosin stabilize the conformation of formin-nucleated actin filaments. J. Biol. Chem. *287*, 31894–31904.
8. Wawro, B., Greenfield, N.J., Wear, M.A., Cooper, J.A., Higgs, H.N., and Hitchcock-DeGregori, S.E. (2007). Tropomyosin regulates elongation by formin at the fast-growing end of the actin filament. Biochemistry *46*, 8146–8155.
9. Gunning, P.W., Schevzov, G., Kee, A.J., and Hardeman, E.C. (2005). Tropomyosin isoforms: divining rods for actin cytoskeleton function. Trends Cell Biol. *15*, 333–341.
10. Balasubramanian, M.K., Helfman, D.M., and Hemmingsen, S.M. (1992). A new tropomyosin essential for cytokinesis in the fission yeast S. pombe. Nature *360*, 84–87.
11. Skoumpla, K., Coulton, A.T., Lehman, W., Geeves, M.A., and Mulvihill, D.P. (2007). Acetylation regulates tropomyosin function in the fission yeast *Schizosaccharomyces pombe*. J. Cell Sci. *120*, 1635–1645.
12. East, D.A., Sousa, D., Martin, S.R., Edwards, T.A., Lehman, W., and Mulvihill, D.P. (2011). Altering the stability of the Cdc8 overlap region modulates the ability of this tropomyosin to bind co-operatively to actin and regulate myosin. Biochem. J. *438*, 265–273.
13. Coulton, A.T., East, D.A., Galinska-Rakoczy, A., Lehman, W., and Mulvihill, D.P. (2010). The recruitment of acetylated and unacetylated tropomyosin to distinct actin polymers permits the discrete regulation of specific myosins in fission yeast. J. Cell Sci. *123*, 3235–3243.
14. Feierbach, B., and Chang, F. (2001). Roles of the fission yeast formin for3p in cell polarity, actin cable formation and symmetric cell division. Curr. Biol. *11*, 1656–1665.
15. Chang, F., Drubin, D., and Nurse, P. (1997). cdc12p, a protein required for cytokinesis in fission yeast, is a component of the cell division ring and interacts with profilin. J. Cell Biol. *137*, 169–182.
16. Mata, J., and Nurse, P. (1997). tea1 and the microtubular cytoskeleton are important for generating global spatial order within the fission yeast cell. Cell *89*, 939–949.
17. Martin, S.G., McDonald, W.H., Yates, J.R., 3rd, and Chang, F. (2005). Tea4p links microtubule plus ends with the formin for3p in the establishment of cell polarity. Dev. Cell *8*, 479–491.
18. Huang, J., Huang, Y., Yu, H., Subramanian, D., Padmanabhan, A., Thadani, R., Tao, Y., Tang, X., Wedlich-Soldner, R., and Balasubramanian, M.K. (2012). Nonmedially assembled F-actin cables incorporate into the actomyosin ring in fission yeast. J. Cell Biol. *199*, 831–847.
19. Karagiannis, J., Bimbó, A., Rajagopalan, S., Liu, J., and Balasubramanian, M.K. (2005). The nuclear kinase Lsk1p positively regulates the septation initiation network and promotes the successful completion of cytokinesis in response to perturbation of the actomyosin ring in Schizosaccharomyces pombe. Mol. Biol. Cell *16*, 358–371.
20. Scott, B.J., Neidt, E.M., and Kovar, D.R. (2011). The functionally distinct fission yeast formins have specific actin-assembly properties. Mol. Biol. Cell *22*, 3826–3839.
21. Grallert, A., Martín-García, R., Bagley, S., and Mulvihill, D.P. (2007). In vivo movement of the type V myosin Myo52 requires dimerisation but is independent of the neck domain. J. Cell Sci. *120*, 4093–4098.
22. Mulvihill, D.P., Barretto, C., and Hyams, J.S. (2001). Localization of fission yeast type II myosin, Myo2, to the cytokinetic actin ring is regulated by phosphorylation of a C-terminal coiled-coil domain and requires a functional septation initiation network. Mol. Biol. Cell *12*, 4044–4053.

23. Stark, B.C., Sladewski, T.E., Pollard, L.W., and Lord, M. (2010). Tropomyosin and myosin-II cellular levels promote actomyosin ring assembly in fission yeast. Mol. Biol. Cell *21*, 989–1000.
24. Galkin, V.E., Orlova, A., Schröder, G.F., and Egelman, E.H. (2010). Structural polymorphism in F-actin. Nat. Struct. Mol. Biol. *17*, 1318–1323.
25. Martin, S.G., and Chang, F. (2006). Dynamics of the formin for3p in actin cable assembly. Curr. Biol. *16*, 1161–1170.
26. Bugyi, B., Papp, G., Hild, G., Lõrinczy, D., Nevalainen, E.M., Lappalainen, P., Somogyi, B., and Nyitrai, M. (2006). Formins regulate actin filament flexibility through long range allosteric interactions. J. Biol. Chem. *281*, 10727–10736.
27. Papp, G., Bugyi, B., Ujfalusi, Z., Barkó, S., Hild, G., Somogyi, B., and Nyitrai, M. (2006). Conformational changes in actin filaments induced by formin binding to the barbed end. Biophys. J. *91*, 2564–2572.