Filament-dependent and -independent Localization Modes of Drosophila Non-muscle Myosin II

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Myosin II assembles into force-generating filaments that drive cytokinesis and the organization of the cell cortex. Regulation of myosin II activity can occur through modulation of filament assembly and by targeting to appropriate cellular sites. Here we show, using salt-dependent solubility and a novel fluorescence resonance energy transfer assay, that assembly of the Drosophila non-muscle myosin II heavy chain, zipper, is mediated by a 90-residue region (1849–1940) of the coiled-coil tail domain. This filament assembly domain, transiently expressed in Drosophila S2 cells, does not localize to the interphase cortex or the cytokinetic cleavage furrow, whereas a 500-residue region in domain. This filament assembly domain, transiently expressed by a 90-residue region (1849–1940) of the coiled-coil tail domain. This filament assembly domain, transiently expressed in Drosophila S2 cells, does not localize to the interphase cortex or the cytokinetic cleavage furrow, whereas a 500-residue region in domain but not the cytokinetic cleavage furrow. Targeting to these two sites appears to utilize distinct localization mechanisms as the assembly domain is required for cleavage furrow recruitment of a truncated coiled-coil tail region but not targeting to the interphase cortex. These results delineate the requirements for zipper filament assembly and indicate that the ability to form filaments is necessary for targeting to the cleavage furrow but not to the interphase cortex.

The motor protein myosin II assembles into bipolar filaments that, through interactions with F-actin, generate contractile forces that are important for many cellular processes. In non-muscle cells, myosin II accumulates at the cytokinetic cleavage furrow with a band of F-actin where it constricts the midzone of the cell to produce two daughter cells (1, 2). Myosin II is also important for organization of the cell cortex in processes such as cell movement (3, 4), cell polarity, and asymmetric cell division (5–9). In this study, we investigated the requirements for assembly of Drosophila myosin II and its targeting to the cortex and cleavage furrow.

Actomyosin contraction requires the assembly of individual myosin II hexamers (containing two heavy chains and two pairs of distinct light chains) into bipolar filaments that drive actin filaments in opposite directions. Bipolar filament assembly is mediated by a large coiled-coil tail domain present in the heavy chain. In myosin IIs from several organisms only a small region of the tail domain is required to confer the ability to assemble into filaments (10–16). However, the best-studied examples of filament assembly, myosin II tail domains from Acanthamoeba and Dicystostelium, show little sequence homology (~10% identity, ~19% homology) to zipper, the Drosophila myosin II heavy chain. Furthermore, the tail domain of zipper displays uniform conservation (~52% identity, ~74% homology) with those of vertebrate non-muscle myosin IIs, which leaves the precise determinants of filament assembly unclear.

As filament assembly is a prerequisite for contractile force generation, modulation of filament stability can be used to regulate actomyosin contraction. Filament stability can be regulated by covalent modification of the tail domain, regulatory light chain, or by ligand binding to the tail domain. In Dictyostelium, myosin heavy chain kinase phosphorylates 3 threonine residues in the tail domain, which leads to inhibition of filament assembly (17, 18). However, myosin heavy chain kinases do not exist in higher organisms and the mechanism of filament assembly regulation is less clear. Although other kinases can phosphorylate the heavy chain, these phosphorylation events appear to be functionally distinct from the myosin heavy chain kinase regulation of Dictyostelium myosin II. For example, although protein kinase C phosphorylates zipper, phosphorylation does not appear to alter filament stability and is not required for viability (19). In cultured mammalian cells however, atypical protein kinase C phosphorylates myosin II, which leads to slower filament assembly and delocalized myosin II (20).

Proper myosin II function also requires recruitment of filaments to specific cellular sites. Myosin II localizes to the F-actin-rich cell cortex, where it alters cellular mechanical properties and actively organizes the molecular composition of the cortex (8). For example, in Caenorhabditis elegans, cortical flows of myosin II are required for the segregation of cell fate determinants in the early embryo (21–23). In the C. elegans one-cell embryo, cortical myosin II forms a dynamic cortical network of contractility that is destabilized near the point of sperm entry. The resulting asymmetry induces cortical flow of myosin II, F-actin, and Par proteins toward the opposite pole. Myosin II localization is also important during mitosis, where it is depleted at the polar cortex and becomes highly concentrated around a narrow band of the cell surrounding the central spindle that ultimately forms the contractile ring (1). Interestingly, in Dictyostelium localization to the cleavage furrow does not require the actin-binding motor domain of myosin II as myosins in which the motor domain has been replaced by green fluorescent protein correctly localize to the cleavage furrow (24). How-
ever, the role of the motor domain in the localization of myosin II in higher organisms is unknown.

To further understand myosin II filament assembly and localization, we have investigated these properties using the *Drosophila* non-muscle myosin II heavy chain, zipper. Zipper function is important for diverse developmental processes, including border cell migration (25), nuclear migration in the syncytial preblastoderm (26), cellularization, and dorsal closure (27–29). During embryonic cellularization the plasma membrane invaginates in the syncytial blastoderm around cortically positioned nuclei to simultaneously form 6000 columnar epithelial cells. Contraction of an actin array by myosin II is required for the basal closure of the membrane around the nuclei (28).

In *Drosophila* neuroblasts, precursors of the central nervous system, zipper function is required for correct partitioning of cell fate determinants in a similar but mechanistically distinct manner as occurs in *C. elegans* (7, 8, 22). Myosin II is initially restricted to the apical cortex by the tumor suppressor Lethal (2) giant larvae (Lgl) (7). During anaphase and telophase, myosin II localization expands basally and this correlates with the basal restriction of cell fate determinants. Although myosin II function at the cell cortex is important in many different contexts, the determinants for its cortical localization are unknown. Here we examine the determinants of zipper filament assembly and localization with the goal of understanding myosin II function at the cell cortex.

**EXPERIMENTAL PROCEDURES**

*Molecular Cloning, Protein Expression, and Purification*—Zipper regions were subcloned using a plasmid containing the full-length zipper B sequence that was kindly provided by Dan Kiehart (19). Zipper regions were expressed in *Escherichia coli* using the pET-19b derivative pBH, which places a hexahistidine purification tag followed by a tobacco etch virus protease cleavage site at the NH2 terminus of the expressed protein (30). Hexahistidine fusion proteins were purified using nickel-nitrilotriacetic acid resin and standard protocols. Ion exchange chromatography was used to further purify proteins if necessary. Purified proteins were dialyzed extensively against salt-free buffer (10 mM Tris, pH 8.0, 1 mM dithiothreitol, 1 mM EDTA) at 4 °C. Purity was established using SDS-PAGE and/or matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

**Assembly Assays**—For the solubility assay, we incubated zipper tail regions at 20 μM at a range of salt concentrations in 10 mM Tris, pH 8.0, 1 mM dithiothreitol, 1 mM EDTA for 30 min at 4 °C followed by centrifugation at 100,000 × g for 30 min. We then separated the soluble and insoluble phases and loaded equal volumes for analysis by SDS-PAGE. We used ImageJ (NIH) to determine relative protein amounts from scanned, Coomassie Brilliant Blue-stained gels.

For the fluorescence resonance energy transfer (FRET)2 assay, we expressed and purified cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) NH2-terminal zipper fusions separately and mixed them in equal molar proportions to a total fluorescent protein-zipper fusion concentration of 20 μM. We then acquired fluorescence emission spectra at a range of salt concentrations on an ISS PC1 spectrofluorometer with an excitation wavelength of 433 nm and a slit width of 0.5 nm. The sample temperature was maintained at 20 °C using a circulating water bath.

**Cell Culture and Transfection**—We transiently transfected *Drosophila* S2 cells grown in Schneider’s insect medium supplemented with 10% fetal bovine serum with the pMT/V5-HisA vector (Invitrogen) containing hemagglutinin (HA) or enhanced green fluorescent protein NH2-terminal fusions to zipper regions. The *Drosophila* embryo-derived Schneider cell line (S2) was maintained in Schneider’s insect medium supplemented with 10% heat-inactivated fetal bovine serum at room temperature. For transient expression of zipper fragments, between 1 and 3.5 million cells at fast growing stage were transfected with 0.8 μg of plasmid DNA using Effectene reagent (Qiagen) in 6-well plates. After 16 h of recovery, cells were induced with 0.5 mM CuSO4 for 16–24 h and subjected to detecting protein expression by Western blot or fixing and immunofluorescence staining.

**Immunofluorescence and Imaging**—To determine localization of endogenous and ectopic zipper, S2 cells were settled down on No. 1.5 coverslip and fixed with 4% paraformaldehyde for 10 min. After fixing, cells were permeabilized and blocked in blocking solution (1× phosphate-buffered saline, 0.1% Triton-X, 1% bovine serum albumin, 10 mM glycine, and 0.02% NaN3), followed by incubations with primary and then secondary antibodies diluted in the blocking solution. Phalloidin-fluorescein isothiocyanate (FITC) (Invitrogen-Molecular Probes) was added to stain F-actin. All procedures were performed at room temperature. The stained cells were mounted in Vectashield with 4’,6-diamidino-2-phenylindole (Vector Laboratories). For drug treatment, cells were transferred to medium containing 20 μM latrunculin A (Sigma) or the parallel Me2SO control for 30 min before fixation. The primary antibodies used include rabbit polyclonal anti-zipper (raised against amino acids 1670–1851) at 1:2500, anti-phosphohistone H3 (Upstate) at 1:500, mouse monoclonal anti-HA (Covance) at 1:1000, and anti-α-tubulin (DM1A; Sigma-Aldrich) at 1:500. The secondary antibodies used included goat anti-rabbit IgG-Cy3, donkey anti-mouse IgG-Cy3 (Jackson ImmunoResearch), and goat anti-rabbit IgG-Alexa Fluor 594 (Invitrogen-Molecular Probes). Images were taken using a Nikon D-Eclipse C1 inverted confocal microscope using either a ×40 air or ×60 oil immersion objective.

**Time-lapse Imaging**—For following localization of green fluorescent protein-tagged zipper tail domain, transfected S2 cells were imaged using a PerkinElmer spinning disk confocal microscope on a Nikon Eclipse TE2000-U inverted microscope. A ×60 oil immersion objective was used to collect 3–5 z steps at 1-μm intervals every 15–60 s using Metamorph software. All data were processed by ImageJ and reconstructed into Quicktime movies.

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2 The abbreviations used are: FRET, fluorescence resonance energy transfer; iCTD, interphase cortical targeting domain; FITC, fluorescein isothiocyanate; CFP, cyan fluorescent protein; YFP, yellow fluorescent protein; HA, hemagglutinin; AD, assembly domain.
Dictyostelium myosin II is highly salt-dependent (13), only domain contains sin II heavy chain, zipper, consists of an 800-residue motor

ment in a salt-dependent manner (Fig. 1
tail domain regions exhibited different propensities to sedi-
unassembled coiled-coil dimers remain in solution (13). Zipper
paracrystals efficiently sediment upon centrifugation, whereas
assays to identify the AD to higher resolution. Filaments and
for the ability to form filaments using salt-dependent assembly

of the tail domain at low, medium, and high salt concentrations. Energy transfer is observed at 75 mM salt where the tail domain is expected to form filaments. F, salt dependence of FRET signal for several tail fragments. Error bars represent S.D. from three independent measurements.

RESULTS

The Zipper Tail Domain Contains a 90-Residue Filament “Assembly Domain”—We first defined the precise require-
ments for oligomerization of the coiled-coil tail so that we could
compare the zipper elements that specify filament assembly
with those that target it to the cell cortex. The *Drosophila* myo-

II heavy chain, zipper, consists of an 800-residue motor
domain and a 1000-residue tail domain (Fig. 1A). The tail
domain contains ~800 residues with a heptad repeat, consist-
ent with its ability to formed coiled-coils, followed by a short
section known as the tailpiece, which lacks the heptad repeat.
Interactions between coiled-coils are responsible for higher
order oligomerization of myosin II into bipolar filaments.

Which elements of the zipper tail sequence are necessary for
tail domain assembly? The so-called “assembly domains” (AD)
for several non-muscle myosin heavy chain tail domains have
been determined (13–16), but the homology among tail
domains is distributed uniformly and in some cases is very low,
such that it is not yet possible to predict the AD of myosin II tail
domains. The zipper AD is known to occur in the COOH-ter-
section known as the tailpiece, which lacks the heptad repeat.

To measure the extent of filament assembly in solution, we
developed an assay based on FRET between NH2-terminal-at-
tached fluorescent proteins. We predicted that the close prox-
imity of the NH2-terminal regions of the tail when polymerized
would allow for energy transfer between fluorescent proteins at
this position (Fig. 1D). A solution of CFP and YFP fusions of
zipper show a salt-dependent ratio of donor (CFP) to acceptor
(YFP) fluorescence, indicative of energy transfer in the filament
state (Fig. 1, D and E).

The FRET and pelleting assays yielded qualitatively similar
results, indicating that the separation between soluble and
insoluble phases that occurs in the pelleting assay did not sig-
nificantly perturb the equilibrium between individual coiled-
coils and higher order oligomers. It should be noted that plac-
ing fluorescent “heads” on *Dictyostelium* myosin II restores the
bipolar mini-filament nature of the oligomers formed by tail
domain truncations (13); thus, the FRET assay likely represents
filaments that are in a native form. Furthermore, identical pel-
leting results were obtained using fluorescent protein-tagged or
non-tagged tail fragments (Fig. 1B). The only exception
occurred when the CFP and YFP were placed directly adjacent
to the assembly domain (see below) where they inhibited fila-
ment assembly, presumably for steric reasons. The FRET assay
did allow for observation of subtle differences between zipper
tail regions in their ability to form filaments. For example, oli-
gomers of the tail domain regions 1744–2011 and 1570–1969
are maximally stable at different salt concentrations, indicating

![Figure 1](https://example.com/figure1.png)

**FIGURE 1. Identification of a zipper filament assembly domain.** A, zipper domain structure. A schematic of a zipper dimer is shown with motor domains (ovals), coiled-coil tail domain (rod; amino acid residue 1111–1969), and non-helical tailpiece (circle). Non-tagged zipper tail domain proteins were used on panels B and C except where indicated, and fluorescent protein-tagged tail domain proteins were used on panels E and F. B, pelleting assay for filament assembly for several tail domain regions. S, supernatant fraction; P, pellet fraction. C, quantification of solubility for tail domain regions shown in panel B. D, scheme for FRET-based assay for filament assembly. No FRET is observed when CFP fusions (dark circles) are dissociated from YFP fusions (light circles). When filament assembly occurs, CFP and YFP are close enough to allow for energy transfer. E, fluorescence emission spectra of an assembly domain-containing zipper tail domain at low, medium, and high salt concentrations. Energy transfer is observed at 75 mM salt where the tail domain is expected to form filaments. F, salt dependence of FRET signal for several tail fragments. Error bars represent S.D. from three independent measurements.
that regions outside the AD can influence filament stability. We expect that this assay will also be useful for measuring other filament assembly parameters, including assembly kinetics.

Comparison of the ability of different regions of the zipper tail to assemble reveals that residues 1849–1940 comprise a minimal AD that is both necessary and sufficient to robustly assemble into filaments (Fig. 2A). Sequence comparison shows that the zipper AD partially overlaps the 39-residue second assembly competence domain identified in human non-muscle myosin (15) and encompasses the 29-residue AD found in sarcomeric myosin II (16). The zipper AD does not include the globular tailpiece, which is required for filament assembly in *Acanthamoeba* but not Dictyostelium myosin IIs (13, 14), vertebrate smooth muscle, and non-muscle myosin II (31). Unlike the Dictyostelium AD, which is far away from the tip of the coiled-coil rod, the zipper AD is close to the end of the rod and does not have “Ala-rich” flanking segments that contain alanines in the core “a” and “d” heptad repeat positions (13).

What are the unique features of the zipper tail assembly domain that allow it to oligomerize? The ability of other segments of the tail domain, such as 1550–1810, to form coiled-coils suggests that a trigger sequence necessary for coiled-coil formation (32) is not the unique feature of the AD. Like other myosin II tail domains, the zipper tail domain contains a repeating charge structure that may be important for specifying the structure of the filament (Fig. 2C) (13, 33). Fig. 2C shows the overall charge of 15- and 29-residue windows of the zipper tail. Although this repeat occurs throughout the tail domain, including in regions that do not assemble into filaments, the assembly domain is the region in which the repeat is most evenly distributed among alternating 15-residue positive and negative charged sequences. Further study will be needed to establish the role of the specific charge repeat in stabilizing the filament structure.

**The Zipper Tail Domain Specifies Cortical Localization in *Drosophila* S2 Cells**—We next examined the localization of myosin II in *Drosophila* S2 cells to delineate the structural requirements for localization. Myosin II exhibits a complex localization pattern in S2 cells (Fig. 3A) that is similar to many other cell types: during interphase, myosin II exists both in the cytoplasm and at the cortex; during mitosis, it is depleted from the cortex until anaphase at which point it forms cortical patches; in late anaphase and telophase, myosin II concentrates in a narrow band around the central spindle, ultimately forming the cytokinetic ring. Thus, cortical localization occurs in interphase, anaphase, and telophase but is inhibited in early mitosis.

We expressed both the motor and tail domains in S2 cells to determine which elements of the heavy chain are required for localization. As shown in Fig. 3B, full-length zipper transfected into S2 cells under the control of an inducible copper promoter exhibits a localization pattern like endogenous zipper. However, the motor domain remains in the cytoplasm throughout the cell cycle (Fig. 3C), indicating that it is not sufficient for targeting to the interphase cortex or cleavage furrow. In contrast, the tail domain does specify cortical localization in both of these contexts (Fig. 3D). As the tail domain is responsible for zipper dimerization, we cannot rule out interactions with endogenous zipper to form mixed coiled-coils as the localization mechanism of the full tail domain based on this data alone. However, several large tail domain regions with many heptad repeats are unable to
Drosophila Myosin II Filament Assembly and Localization

The Zipper Filament Assembly Domain Is Not Recruited to the Cortex—To determine the elements of the tail domain required for cortical targeting, we first examined the localization properties of the AD as it is recruited to the cleavage furrow in Dictyostelium. Interestingly, we find that the AD (1849 – 1940) is unable to be targeted to either the interphase cortex or the cytokinetic cleavage furrow in S2 cells but instead remains in the cytoplasm throughout the cell cycle (Fig. 3E), although it is expressed properly, as assayed by anti-HA Western blotting (Fig. 3F). The inability of the filament AD to localize indicates that either it does not form filaments in the cell or AD filaments cannot target to the cortex.

Interphase Cortical Localization Does Not Require the Assembly Domain—As the assembly domain is not recruited to the cell cortex, we examined other tail domain regions for their ability to localize to the interphase cortex and the cytokinetic cleavage furrow. Analysis of a series of tail domain regions reveals that the ability to form filaments in the AD is almost entirely distinct from the filament assembly domain. Although many tail domain regions remained in the cytoplasm, those that contain residues 1350–1865 efficiently localized to the cell cortex during interphase (Fig. 4A). The iCTD only partially overlaps with the AD, indicating that the AD, and presumably the ability to form filaments, is dispensable for localization to the interphase cortex.

Truncation of the iCTD at the NH2 terminus causes the tail domain to localize less efficiently, such that by residue 1670 it is predominantly cytoplasmic (1670–1865); however, intermediate length tail domain regions such as 1500–1865 still show partial cortical localization (Fig. 4A) when each is transfected at ~20% efficiency and expressed at roughly equivalent levels (Fig. 4B). The COOH-terminal iCTD boundary is more defined, such that truncation from residue 1865 to residue 1851 is sufficient to abolish cortical localization. As large regions of the tail domain were unable to localize to the cortex, we conclude that interaction with endogenous zipper (through mixed coiled-coils) is not sufficient to specify localization.

Although the iCTD region of the zipper tail domain efficiently localizes to the cortex during interphase, it is not recruited to and enriched at the cleavage furrow during cytokinesis (Fig. 4C and supplemental Movie S1). Early in mitosis, the strong cortical localization of the iCTD is lost (supplemental Movie S2) and becomes predominantly cytoplasmic, where it remains until the end of cytokinesis.

A hallmark of the cell cortex is the presence of dense meshwork of F-actin below the cell membrane. To test whether cortical F-actin is required for iCTD localization, we treated S2 cells with latrunculin A. As shown in Fig. 4D, treatment with latrunculin A (Lat A) leads to loss of cortical F-actin as assayed by a loss of FITC-phalloidin staining. However, zipper iCTD proteins are still targeted to the cortex despite the loss of F-actin. Thus, the zipper iCTD cortical anchor is not F-actin and is not dependent on the presence of F-actin.

When the ability to form filaments is restored to the iCTD by adding the AD, localization occurs predominantly to the cortex, but rather than the uniform distribution observed with the iCTD alone, localization is in cortical patches (Fig. 4, E and F).
As the patches correlate with the presence of the AD, these discrete structures may result from assembly of the tail region into filaments. In addition, they appear similar to the cortical patches of the *C. elegans* heavy chain NMY-2 in the early embryo.

**Cleavage Furrow Localization Requires the Filament Assembly Domain**—We next asked what region of the tail domain is sufficient for cleavage furrow localization as the iCTD becomes cytoplasmic during mitosis. As expected, the tail domain fragment 1350–1940 (iCTD/H11001 AD) localizes to the cleavage furrow like the full-length zipper during mitosis (Fig. 5A). Truncation of the zipper tail domain at the NH$_2$ terminus does not inhibit furrow-targeting ability until residue 1744 (Fig. 5E). Regions that encompass the AD, and ~100 more residues (1744–1969) that constitute a furrow-targeting domain (Fig. 5D), efficiently localize to the cleavage furrow during cytokinesis (Fig. 5 and supplemental Movie S3). As this region lacks the complete iCTD, it fails to target to the cortex during interphase but does form cytoplasmic aggregates, consistent with its ability to form filaments (Fig. 5B). Interestingly, a region of the tail that includes the furrow-targeting domain and the non-helical tailpiece (1744–2011) localizes to the cytoplasm but fails to form aggregates, indicating that this region may be involved in filament assembly regulation (Fig. 5C). We observed cortical puncta of fragment 1350–1940 (iCTD + AD) throughout the cell cycle (Fig. 5A), rather than only at late metaphase and anaphase like endogenous myosin II (Figs. 3A and 5A) either because of increased protein level or misregulation due to the presence of the iCTD but lack of head and light chain binding domains.

**DISCUSSION**

Myosin II undergoes dynamic filament assembly and localization during cell division and other processes. Although the molecular mechanism of these dynamics is fairly well understood in *Dictyostelium*, it is less clear in higher organisms that lack myosin heavy chain kinase. To contribute to our understanding of myosin II filament assembly and localization in higher organisms, we have examined the filament assembly and localization properties of the *Drosophila* non-muscle myosin II.

**Filament Assembly Determinants**—Of the >1000-residue zipper tail domain, only a 90-residue segment is required to assemble into oligomers that are disrupted by high salt, similar to filament assembly domains from various organisms (11, 13–15, 31). The 90-residue zipper AD does not include the non-helical tailpiece and is close to the end of coiled-coil rod.
FIGURE 5. The zipper cleavage furrow-targeting domain. A, selected time point of time-lapse imaging of green fluorescent protein-tagged full-length zipper 1–2011 and tail domains 1350–1940 and 1744–1969. The time is shown as mins, and zero time point is the onset of DNA segregation (supplemental movies S3–S5). B, a region of the zipper tail that includes the filament assembly domain specifies cleavage furrow targeting. Transiently transfected zipper 1744–1969 and mitotic marker, visualized by anti-HA and anti-phospho-histone H3 (PH3) antibody staining, respectively, are shown in cells at different cell cycle stages. C, the non-helical tailpiece disrupts cytoplasmic aggregation of the cleavage furrow-targeting domain. The tail domain region 1744–1969 forms cytoplasmic aggregates, but the same region that also includes the non-helical tailpiece (1744–2011) does not. Zipper tail domains and F-actin are shown with anti-HA and FITC-phalloidin staining. D, the relative positions of interphase cortical targeting domain (iCTD), assembly domain (AD), and furrow-targeting domain (FTD) located in the zipper tail region. E, cleavage furrow targeting requires AD. Anaphase cellular localization of different tail domain constructs is shown by anti-HA staining. The mitotic marker PH3 staining is not displayed, but the spindle poles are clear in cells in the lower panel. Scale bar, 3 μm.

(Fig. 2A). Based on sequence comparison, the zipper AD encompasses the assembly competence domain (AD) of sarcomeric myosin II and overlaps the second assembly competence domain of human non-muscle myosin IIb, but is distinct from the region that is critical for assembly of vertebrate smooth muscle myosin II (31). There is low sequence homology between zipper and Acanthamoeba or Dictyostelium myosin II heavy chain tail domain beyond the presence of a heptad repeat. Different models have been proposed to illustrate the specific domain associated with filament assembly. Acanthamoeba myosin II heavy chain required the COOH-terminal 14-heptad repeats plus the tailpiece to initiate bipolar filament assembly through hydrophobic as well as electrostatic interactions (14). The AD of Dictyostelium myosin II heavy chain is a 35-residue region within an extended assembly domain flanked by two regions containing alanines in core “a” and “d” heptad positions, with phosphorylation of 3 threonine residues controlling filament formation by folding back the tail (11, 13). The 29-residue AD of vertebrate sarcomeric myosin II has the characteristic of clustered negatively charged residues in the center flanked by positively charged residues on each side (16). Negatively charged and positively charged assembly competence domains from human non-muscle myosin IIB are located 100 residues apart, and antiparallel electrostatic interactions between these domains are essential for filament nucleation (15). The Drosophila zipper AD identified in this study possesses an evenly distributed alternating charge repeat and is necessary and sufficient to oligomerize in vitro (Fig. 2).

The lack of a consensus filament assembly model among different myosin IIs may be due to the considerable diversity in filament properties essential for the wide range of cellular activities within various cell types. However, investigation of the interaction between identified assembly domains and other interacting sites should reveal the general principles of filament assembly. The zipper tail domain possesses an alternating charge repeat that is thought to be important for filament assembly. This charge repeat is more evenly distributed in the AD than the rest of the tail, which fails to assemble into filaments (Fig. 2C). We are currently undertaking a mutational analysis to test the importance of the charge repeat in filament stability.

Localization Determinants—We have found that interphase and mitotic cortical localization in S2 cells requires distinct tail domain elements. In interphase S2 cells, myosin II partitions between cortical and cytoplasmic pools, but the targeting domain that we have identified (iCTD) is biased toward cortical localization (Figs. 3, A and B, and 4, A and C). This suggests that cortical targeting is regulated during interphase. The iCTD does not contain the filament AD so we expect that it does not form filaments in the cell. Thus, at least one mode of interphase cortical targeting utilizes a mechanism that does not require filament assembly. However, when the iCTD is combined with the filament AD, the localization becomes punctate rather than the even distribution observed with the iCTD alone (Fig. 4F). It has been observed that deletion of the AD of α-cardiac MYH expressed in COS cells abolished the formation of a needle-
shaped structure (16). Surprisingly, inclusion of the non-helical tailpiece causes this punctate localization to be lost, although we observed no effect of the tailpiece on the in vitro filament assembly characteristics (Figs. 1B and 5C). If punctate localization represents filament assembly, which is supported by the correlation of punctate localization with the presence of the AD, then the tailpiece may be involved in regulating filament assembly.

Myosin II localization becomes very dynamic during mitosis, and these dynamics appear to be important for function. For example, photobleaching experiments in Dictyostelium have shown that myosin II dynamically cycles between the cytoplasm and furrow and that this cycling requires filament assembly and disassembly (34). In S2 cells, myosin II is highly regulated (35) and is not detected on the cortex early in mitosis but forms dynamic cortical aggregates at the metaphase-to-anaphase transition and ultimately forms a concentrated band around the central spindle that becomes the cleavage furrow (Figs. 3A and 5A). The initial recruitment of myosin II to the F-actin-rich equatorial cortex requires myosin regulatory light chain phosphorylation through Rho1 signaling (35, 36). Because the recruitment of myosin to the equatorial cortex during mitosis is independent of F-actin, it is likely that the role of regulatory light chain is to regulate myosin II filament assembly rather than its actin-dependent ATPase activity. Our data show that non-muscle myosin II filament formation is essential for cleavage furrow localization and requires the zipper AD but localization to the interphase actin cortex is independent of filament assembly (Figs. 4 and 5). Consistent with this, the AD of vertebrate smooth muscle and human non-muscle myosin IIa is required for furrow localization in COS cells (31).

Surprisingly, the minimum filament AD (1849–1940) that forms filaments in vitro fails to localize to the cortex at any stage of the cell cycle (Fig. 3E). However, a slightly larger region (1744–1969), the furrow-targeting domain, efficiently localizes to the cleavage furrow (Fig. 5, A and B). It is unlikely that these tail fragments localize to the cleavage furrow through mixed coiled-coils with endogenous myosin since several long tail domain fragments, including the iCTD, were not recruited to the cleavage furrow. However, based on our data, we cannot rule out the possibility that these tail fragments are recruited to the cleavage furrow through oligomerization with endogenous myosin II.

What is the cortical anchor that recruits myosin II? Few proteins have been identified that bind the heavy chain tail domain. Possible cortical anchors include membrane phospholipids, as the tail domains of mammalian non-muscle myosin IIs have been shown to bind phosphorylserine-containing liposomes (37). Another candidate is the tumor suppressor Lethal (2) giant larvae (Lgl), which binds the myosin II tail domain and is cortically associated with the cytoskeleton (38). Given that we have observed different requirements for interphase and mitotic cortical localization, it is likely that cortical recruitment in these two contexts utilizes distinct anchoring mechanisms. Future work will be directed at the identification of the cortical anchoring factors and possible filament regulatory mechanisms. The reagents described here should be useful in this regard.

Acknowledgments—We thank Michelle Jin for help at the inception of this project. We thank C. Doe for critical reading of the manuscript and members of the Prehoda laboratory for helpful comments and suggestions.

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