

# Revolving movement of a dynamic cluster of actin filaments during mitosis

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The actin cytoskeleton undergoes rapid changes in its architecture during mitosis. Here, we demonstrate novel actin assembly dynamics in M phase. An amorphous cluster of actin filaments appears during prometaphase, revolves horizontally along the cell cortex at a constant angular speed, and fuses into the contractile ring after three to four revolutions. Cdk1 activity is required for the formation of this mitotic actin cluster and its

revolving movement. Rapid turnover of actin in the filaments takes place everywhere in the cluster and is also required for its cluster rotation during mitosis. Knockdown of Arp3, a component of the actin filament-nucleating Arp2/3 complex, inhibits the formation of the mitotic actin cluster without affecting other actin structures. These results identify Arp2/3 complex as a key factor in the generation of the dynamic actin cluster during mitosis.

## Introduction

Cell morphological changes during mitosis are accompanied by dynamic rearrangements of the actin cytoskeleton. Actin filaments are found beneath the cortical plasma membrane and in the retraction fibers during early M phase, and also in the contractile ring at the equatorial region during late M phase. The cortical flow (Bray and White, 1988), which is generated by the interaction of the cortical actin filaments with myosin, is shown to be required for the proper centrosome separation and positioning (Rosenblatt et al., 2004). Moreover, the cortical actin filaments play an important role in the orientation of the mitotic spindle (Théry et al., 2005; Toyoshima and Nishida, 2007).

How the cortical actin filaments are rearranged at the onset of M phase has remained unclear. Small GTPases (Etienne-Manneville and Hall, 2002; Maddox and Burridge, 2003; Dao et al., 2009) have been shown to regulate cell rounding. Furthermore, dMoesin regulates the rearrangement of cortical actin filaments during mitosis, which is important for cortical stiffening (Carreno et al., 2008; Kunda et al., 2008). AIP and cofilin are also involved in the cell rounding (Fujibuchi et al., 2005). Although many players have been identified, the detailed dynamics and mechanisms for actin rearrangements during mitosis have not been fully elucidated.

Here, we find a novel phenomenon of actin assembly dynamics during mitosis: formation of an amorphous actin cluster and its revolving movement. Our analyses demonstrate that Arp2/3 is essential for this dynamic actin cluster.

## Results and discussion

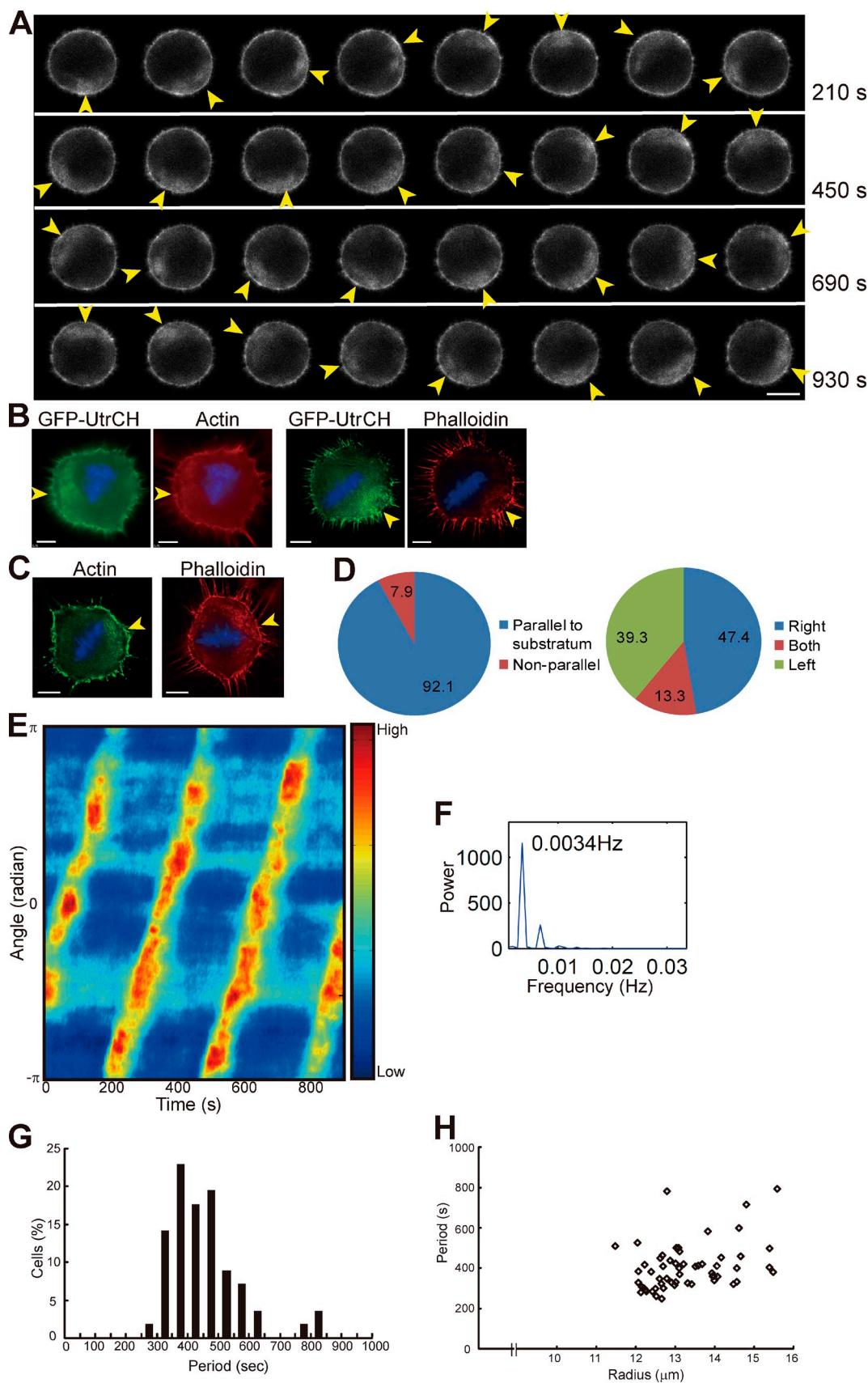
### An amorphous cluster of actin filaments is formed and revolves during mitosis

To examine actin dynamics in living cells, we expressed a calponin homology (CH) domain of utrophin fused to GFP (GFP-UtrCH; Burkhardt et al., 2007; Woolner et al., 2008; Miller and Bement, 2009), which binds to actin filaments and has been used to visualize actin filaments in living cells. Time-lapse observations in HeLa cells have unexpectedly revealed that an amorphous cluster of GFP-UtrCH appears outside the nucleus during prometaphase, and it moves around along the cell cortex at a roughly constant speed until telophase (Fig. 1 A and [Video 1](#)). This actin cluster underwent changes in its shape and size during the revolving movement. Staining with phalloidin and anti-actin antibody indicated that the cluster of GFP-UtrCH consists of F-actin (Fig. 1 B). Observations in cells expressing

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Abbreviation used in this paper: CH, calponin homology.

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**Figure 1. An amorphous cluster of actin filaments revolves along the cell cortex.** (A) Time-lapse images of HeLa cells expressing GFP-UtrCH and DsRed-histone H1 during metaphase. GFP-UtrCH images were taken every 5 s, and images are shown at 30-s intervals. (B) Staining with anti-actin antibody (left) or Alexa Fluor 546–phalloidin (right) of HeLa cells expressing GFP-UtrCH. (C) Staining with anti-actin antibody (left) or Alexa Fluor 546–phalloidin (right) of

GFP-actin have also revealed the formation of an amorphous cluster of F-actin and its revolving movement during mitosis (Fig. S1, A and B). Moreover, staining of control HeLa cells, which do not express exogenous proteins, showed that an amorphous cluster, which is stained with both phalloidin and anti-actin antibody, exists along the cell cortex during prometaphase to anaphase, and that this actin cluster resembles, in its location and shape, the actin cluster visualized with GFP-UtrCH or GFP-actin (Fig. 1 C). Thus, the actin cluster, which is visualized with GFP-UtrCH or GFP-actin, is not an artifact resulting from their overexpression. Collectively, these results show that an amorphous cluster of actin filaments is formed during early prometaphase, and it revolves along the cell cortex until anaphase in HeLa cells.

Time-lapse observations of 146 mitotic cells with GFP-UtrCH demonstrated that every cell exhibited the formation of an amorphous actin cluster, which always revolved during M phase, and that the plane of revolving movement, for the most part (>90%), was oriented parallel to the substrate surface; i.e., the actin cluster revolved horizontally (Fig. 1 D, left). Once the cluster started to revolve, it did not change the direction of movement in most cells; the direction was about half clockwise and half counterclockwise (Fig. 1 D, right). In rare occasions (~13%; both in Fig. 1 D, right), however, changes in direction occurred during the movement. A spatiotemporal representation of the actin cluster movement (Figs. 1 E and S1 C) demonstrates a revolving movement with constant angular velocity in each cell, and its Fourier transformation (Fig. 1 F and S1 D) indicates that the mean value of frequency =  $0.0026 \pm 0.00062$  Hz (period of  $404 \pm 117$  s,  $n = 57$ ; Fig. 1 G). The angular velocity varied slightly from cell to cell, but was not highly dependent on the cell size (correlation coefficient, 0.361; Fig. 1 H). Thus, we could see approximately three to four revolutions during M phase.

#### Cdk1 activity is required for the actin cluster formation and revolving movement

Phalloidin staining in cells with no exogenous proteins has shown that an amorphous cluster of actin filaments is detected during prometaphase to anaphase, but not in G2 phase, prophase, telophase, or G1 phase (Fig. 2, A and C). This is in good agreement with observations with GFP-UtrCH, which show that an amorphous actin cluster appears during early prometaphase and disappears during anaphase to telophase (Fig. 2, B and C). A close examination of time-lapse images suggests that the amorphous cluster fuses into the contractile ring during telophase (Fig. 2 D). Thus, this actin dynamic is an M phase-specific event. We then examined the effect of the addition of the inhibitors of mitotic kinases during prometaphase on the actin cluster revolving movement. Although inhibitors of Aurora B and Plk-1 did not affect the actin cluster dynamics

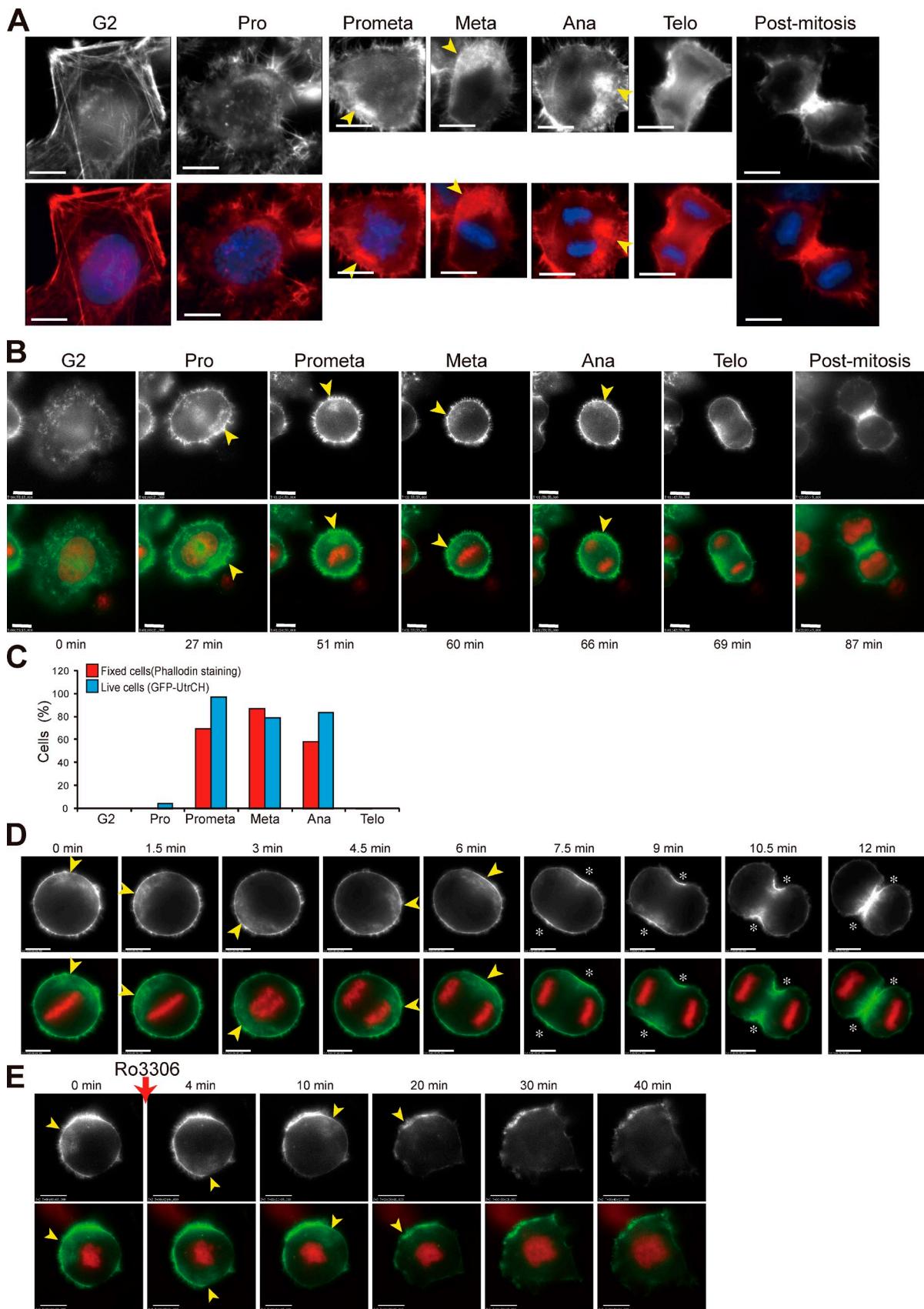
at all (unpublished data), Cdk1 inhibition by Ro3306 (Vassilev et al., 2006) resulted in the disappearance of the actin cluster, which suggests the requirement of Cdk1 activity for the actin cluster dynamics. However, as the inhibition of Cdk1 activity during prometaphase induces the cleavage furrow by promoting M phase exit, the result might not necessarily demonstrate the Cdk1 requirement for the actin dynamics. Then, to prevent the cleavage furrow formation, we treated cells with nocodazole to arrest cells in prometaphase. The treatment did not inhibit the actin cluster formation and movement. In these cells, the addition of Ro3306 resulted in nearly complete disappearance of the actin cluster within 20–30 min (Fig. 2 E and Video 2). As Cdk1-dependent phosphorylation of various proteins (Fig. S2) as well as several Cdk1-dependent events, such as chromosome condensation and cell rounding, also diminished within 15–30 min, it is likely that Cdk1 activity is required for the actin cluster formation and its revolving movement.

#### Cell rounding and cell substratum adhesion are important for the actin cluster formation and revolving movement

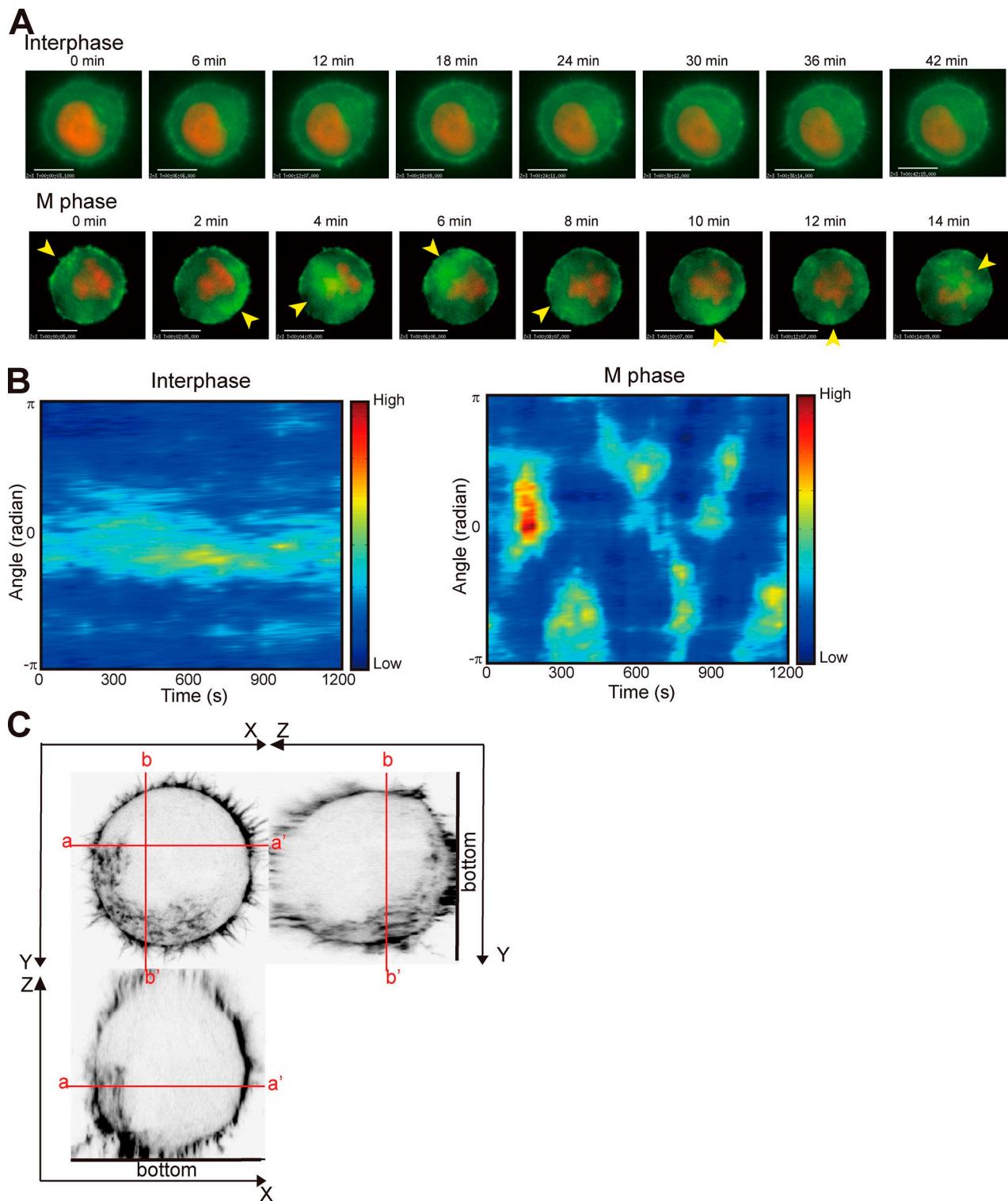
Time-lapse observations in various cell lines showed that formation of a single amorphous actin cluster and its revolving movement, which are similar to those in HeLa cells, occurred during M phase in MCF-7, HepG2, and Cos1 cells (Video 3 and unpublished data), whereas no obvious actin cluster formation and movement occurred in NIH3T3, MDCK, and C3H10T1/2 cells (unpublished data). We noted that those cells that exhibit these dynamic actin behaviors show almost complete cell rounding during M phase, whereas the cell shape of those cells that do not exhibit these actin behaviors is far from globular even in M phase. Of note, these actin behaviors can only be observed in M phase, when cells are rounding. Therefore, there is a correlation between the extent of cell rounding and the occurrence of these dynamic actin behaviors in M phase. In good agreement with this idea, in HaCaT, HEK293T, A431, and KB cells, which show incomplete cell rounding during M phase, the actin cluster formation and movement were also incomplete; i.e., formation of a single actin cluster was not clearly observed, but regions of higher densities of actin filaments were formed in early M phase, and they moved around incompletely along the cell cortex (Video 4 and unpublished data).

Next, we tested the possibility that cell rounding is sufficient to induce the actin cluster dynamics. We cultured HeLa cells in 3D by totally embedding the cells in a gel of reconstituted basement membrane matrix. Under the 3D culture conditions, HeLa cells are round throughout the cell cycle (Fig. 3 A). Time-lapse observations of cells with GFP-UtrCH showed that some heterogeneous distribution of actin filaments could be detected even in interphase, but this heterogeneous distribution pattern did not vary appreciably with time (Fig. 3, A and B, interphase).

HeLa cells. Arrowheads indicate an amorphous cluster of actin filaments. (D) Direction of the revolving movement of the amorphous actin cluster ( $n = 146$ ). (E) Spatiotemporal representation of the revolving movement of the amorphous actin cluster of A. Intensities of GFP-UtrCH signals in areas between the 0.70–0.75 radius away from the center of the cell were averaged and plotted. (F) Fourier transformation of E. (G) Histogram of the period of the revolving movement of the cluster ( $n = 57$ ). (H) Relationship between the radius of cells and the period of the revolving movement of the actin cluster ( $n = 57$ ). Bars: (A) 10  $\mu$ m; (B and C) 5  $\mu$ m.



**Figure 2. Cdk1 activity is required for the actin cluster formation and revolving movement.** (A) Staining with phalloidin of HeLa cells at the indicated phases (G2 to post-mitosis phase). Arrowheads, the cluster of actin filaments. (B) Time-lapse images of HeLa cells expressing GFP-UtrCH and DsRed-histone H1 from G2 to post-mitosis phase. Images were taken every 3 min. Arrowheads, the cluster of actin filaments. (C) Red bars represent the percentages of



**Figure 3. Cell rounding and cell-substratum adhesion are important for the revolving movement of the actin cluster.** (A) Time-lapse images of HeLa cells expressing GFP-UtrCH and DsRed-histone H1 during interphase (top) and M phase (bottom) under the 3D condition. Images were taken every 1 min or 30 s, respectively. The pictures of interphase or M phase, at every 1 min or 2 min, respectively, are shown. Arrowheads indicate the actin cluster. Bar, 10  $\mu$ m. (B) Spatiotemporal representation of GFP signals of A. (C) Confocal images of cross sections of living HeLa cells expressing GFP-UtrCH.

cells containing the cluster of actin filaments stained with phalloidin ( $n = 50$  at each phase). Blue bars represent the percentages of cells exhibiting a revolving actin cluster visualized by GFP-UtrCH ( $n = 50$ ). (D) Close examination of time-lapse images of HeLa cells expressing GFP-UtrCH and DsRed-histone H1. Asterisks indicate the region of the cleavage furrow. Images were taken every 30 s and images are shown at 1.5-min intervals. Arrowheads indicate the actin cluster. (E) Nocodazole-treated, prometaphase-arrested HeLa cells expressing GFP-UtrCH and DsRed-histone H1 were treated with 20  $\mu$ M Ro3306 immediately after time 0. Images were taken every 2 min. Arrowheads, the actin cluster. Bars: (A, D, and E) 10  $\mu$ m; (B) 5  $\mu$ m.

During M phase, however, a relatively large amorphous cluster of actin filaments, within which there were several regions of higher densities of actin filaments, appeared outside the nucleus and moved around along the cell cortex, similar to the amorphous actin cluster under the 2D conditions (Fig. 3, A and B, M phase; and **Video 5**). The orientation of the plane of revolving movement under the 3D conditions, however, shows a random distribution from parallel to vertical, and it varies even during the revolving movement. These results indicate that cell rounding alone is unable to induce the actin cluster formation and revolving movement, and that cell substratum adhesion should be important for determining the orientation of the plane of revolving movement, as these results can be interpreted as the plane of revolving movement being oriented parallel to the cell substratum adhesion surface. Remarkably, x-y, y-z, and x-z images of HeLa cells with GFP-UtrCH cultured in normal 2D conditions demonstrate that densities of actin filaments in the cluster become higher in the bottom part (Fig. 3 C), and this may suggest that most of actin filaments in the cluster originate from the bottom part of the cell, which adheres to the substratum. Thus, cell–substratum adhesion should be important for the actin cluster formation and revolving movement.

#### A rapid actin turnover in the actin cluster

The mitotic amorphous actin cluster undergoes changes in its shape and size continuously (Fig. 1 A), which suggests that actin polymerization and depolymerization actively occur in the cluster. Treatment with the F-actin–depolymerizing drugs latrunculin B and cytochalasin D or the F-actin–stabilizing drug jasplakinolide resulted in the rapid cessation of the revolving movement and the disappearance of the actin cluster (**Fig. S3**). This indicates that actin polymerization and depolymerization reactions are required for generating the mitotic actin cluster and movement. We then performed a FRAP experiment. After photobleaching in the actin cluster, a rapid fluorescent recovery of GFP-actin was observed with a half-life ( $\tau_{1/2}$ ) of  $10.6 \pm 5.9$  s (Fig. 4, A and B; and **Video 6**), which indicates a rapid turnover of actin in the cluster. The fluorescent intensity was restored to almost the original level (Fig. 4 B). Of note, we did not observe any forward or backward movement of the bleached area in the migrating actin cluster (Fig. 4 A). These results suggest that the actin cluster movement is not a movement of stable actin filaments but is generated by a rotational movement of the site with the high actin polymerization activity, which may locate in the cell cortex on the cell–substratum adhesion surface. In other words, a zone, in which actin polymerization and depolymerization occur actively, should revolve in the bottom part of the cell, generating a wavelike movement of an amorphous actin cluster during M phase, like an audience raising their hands to generate a wave in a baseball stadium (Fig. 4 C).

#### Arp2/3 complex is essential for the actin cluster formation and revolving movement

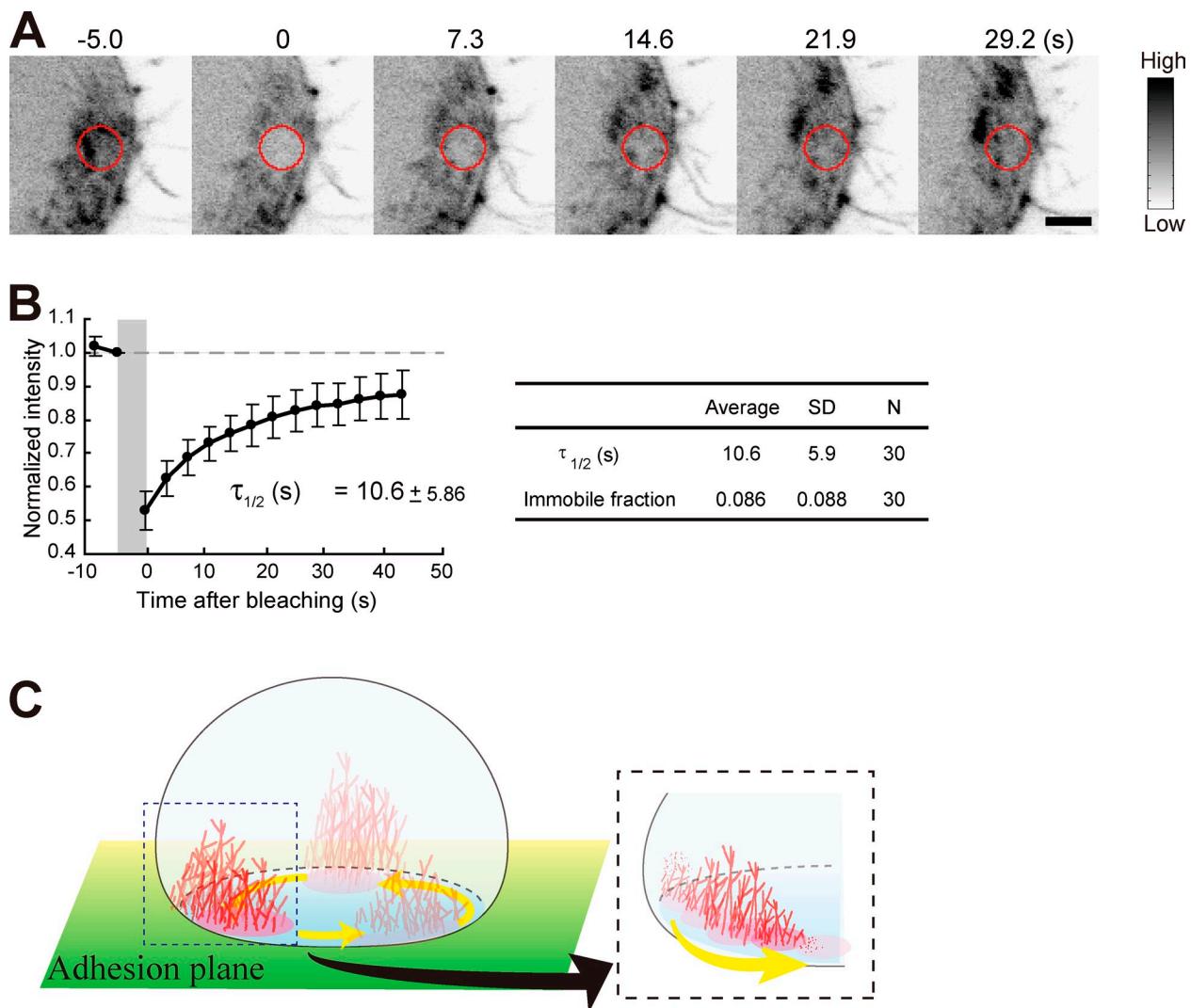
Our results thus indicated the importance of actin-nucleating activity in the mitotic actin cluster dynamics. To identify a factor responsible for the actin cluster formation, we performed RNAi knockdown of actin-nucleating factors (Pollard, 2007;

Chesarone and Goode, 2009). Although knockdown of mDia1, mDia2, or spire had no effect on the actin cluster (unpublished data), knockdown of Arp3, a component of Arp2/3 complex, completely inhibited the actin cluster formation (Fig. 5, A and B). Consistent with previous studies (Steffen et al., 2006; Gomez et al., 2007), Arp3 knockdown resulted in concomitant reduction of Arp2 and Arp2C2, two other components of Arp2/3 complex (Fig. 5 A). Two different siRNA sequences against Arp3, which down-regulated Arp3, gave essentially the same results. Moreover, expression of Arp3res, which is resistant to the siRNA but encodes for wild-type Arp3 protein, in the Arp3 siRNA-transfected cells resulted in the recovery of the actin cluster formation and movement (Fig. 5, C and D). Remarkably, knockdown of Arp3 did not significantly affect the cortical actin structures and retraction fibers (Fig. 5 E), which indicates that the Arp2/3 complex is specifically involved in the generation of the revolving actin cluster during mitosis.

We then determined the subcellular localization of Arp3. Although dense staining in the cytoplasm with anti-Arp3 antibody hindered detection of specific localization of Arp3 in ordinarily prepared samples, the patchy staining of Arp3, which roughly coincided with the amorphous actin cluster and the cortical structures, was clearly detected in permeabilized cells (Fig. 5 F). The area showing higher densities of the patchy spots of Arp3 staining coincided with the amorphous actin cluster area, which was revealed by GFP-UtrCH (Fig. 5 G). The patchy Arp3 staining disappeared in Arp3 siRNA-treated cells (Fig. 5 F), confirming the specificity of Arp3 staining. Moreover, neither patchy staining nor colocalization with the actin cluster was observed in the staining of the same samples with an antibody against Arp1 $\alpha$ , which has been shown to localize in the spindle (Fig. 5 F; Clark and Meyer, 1999). In addition, the Arp3 siRNA did not affect the staining with the anti-Arp1 $\alpha$  antibody, confirming the specificity of the Arp3 siRNA. These results suggest that Arp3 specifically resides in the mitotic actin cluster.

How does the mitotic actin cluster revolve? Our analyses suggest a model in which a zone, where actin polymerization and depolymerization occur actively, revolves in waves along the cell cortex (Fig. 4 C). Because actin polymerization and depolymerization occur actively everywhere in the cluster, but not in specific sites such as the forward or backward area of the direction of revolving movement, the unidirectional actin polymerization may not be a driving force. The cell cortex has been shown to flow from the polar regions of the cell to the equator during M phase. This is called “cortical flow,” and was shown to be inhibited by inhibition of myosin II (Rosenblatt et al., 2004). However, inhibition of myosin II did not inhibit the actin cluster revolution (unpublished data), which suggests that neither cortical flow nor myosin II plays a role in the mitotic actin cluster dynamics. Therefore, elucidation of molecular mechanisms of the mitotic actin cluster dynamics should await further studies.

Murthy and Wadsworth (2008) found that a movement of wavelike changes in GFP-actin fluorescence can be observed in LLC-Pk1 cells only when astral microtubules are disrupted by nocodazole. As we can clearly detect the revolving actin cluster in both intact cells and nocodazole-treated cells, whether the same mechanisms underlie these two apparently similar phenomena



**Figure 4. Actin dynamics in the actin cluster.** (A) Time-lapse confocal images of HeLa cells expressing GFP-actin during prometaphase. Images were taken every 7.3 s. At time 0, photobleaching was performed in the area shown by the red circle. Bar, 5  $\mu$ m. (B) Quantification of FRAP analysis ( $n = 30$ ). Error bars indicate SD. (C) A model of the amorphous actin cluster movement during mitosis. A region (pink) in the bottom part of the cell indicates a zone in which actin polymerization and depolymerization occur actively. Red, actin filaments; yellow, the direction of the revolving movement.

should be examined in future studies. A filamentous actin mesh was observed in starfish oocytes (Lénárt et al., 2005). A cloud of dynamic actin filaments (Li et al., 2008) or filamentous actin meshes (Azoury et al., 2008) were also found in mouse oocytes. Although these mouse structures were shown to depend on formin2, the revolving actin cluster in this study depends on Arp2/3, but not on mDia1 and -2, other formin family members. It will be interesting to see whether these structures share common molecular mechanisms.

What is the physiological role of the revolving movement of the mitotic actin cluster? Arp2/3 has been shown to be required for cell division in yeast (Winter et al., 1997), and a *wsp1* (Wiskott–Aldrich syndrome protein [WASP] is an Arp2/3 activator) mutant of *C. elegans* shows a partial cytokinesis defect (Withee et al., 2004). Moreover, the forced activation of the Arp2/3 complex was reported to cause the delay in M phase progression and the increase in the number of multinucleic cells (Moulding et al., 2007). Thus, it is possible that the revolving

actin cluster, which is generated by Arp2/3-mediated actin nucleation, plays a role in controlling cell division. However, there was also a report showing that depletion of the Arp2/3 complex has no effect on cytokinesis (Bompard et al., 2008). The next challenge is to identify physiological roles of the revolving movement of the mitotic actin cluster.

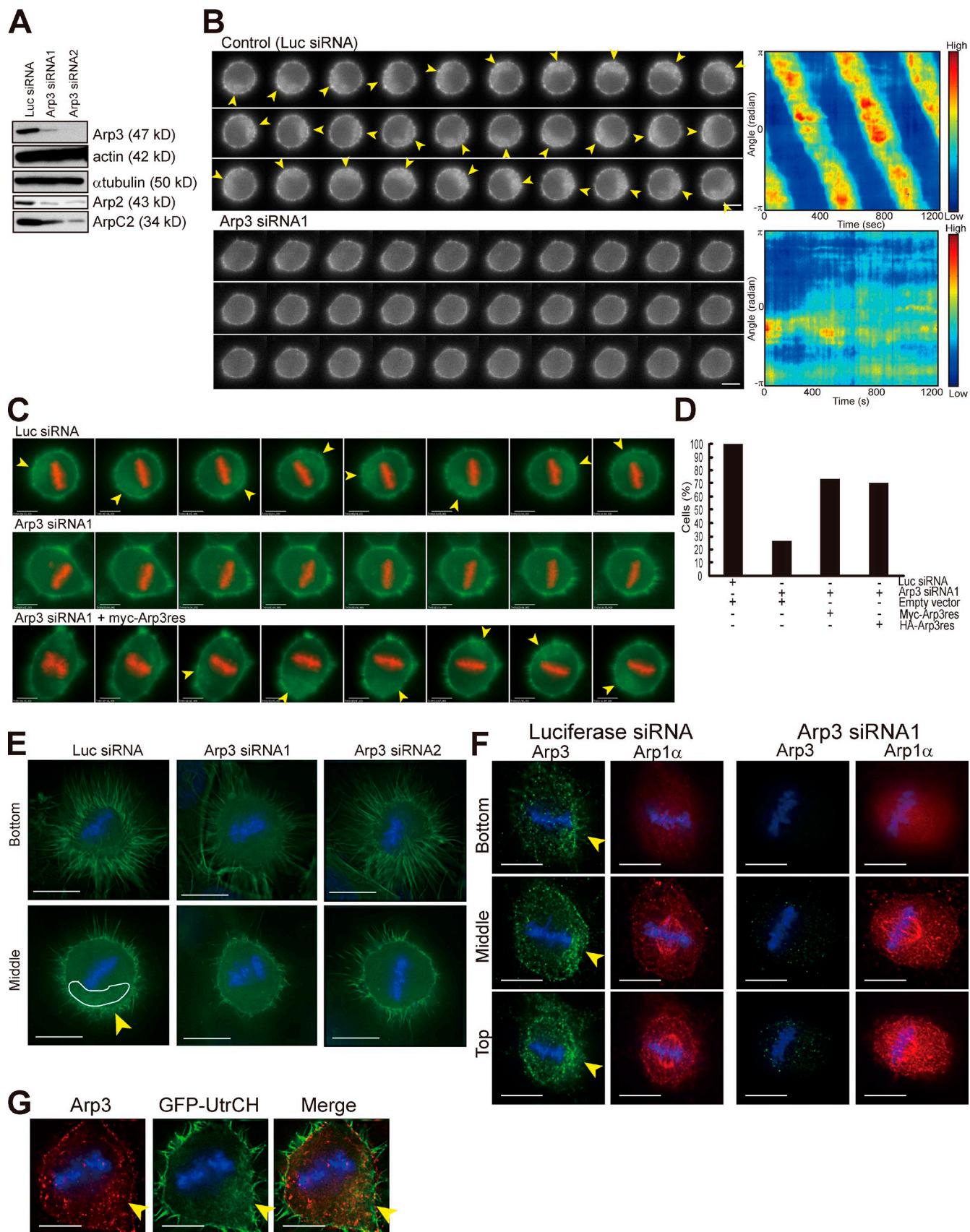
## Materials and methods

### Reagents

Cytchalasin D was obtained from Sigma-Aldrich, and latrunculinB, jasplakinolide, nocodazole, and Ro-3306 were obtained from EMD. The following antibodies were used: anti-actin (AC15), anti- $\alpha$ -tubulin (DM1A), and anti-Arp3 (FMS338; Sigma-Aldrich); anti-cyclinB1 and anti-cMyc (9E10; Santa Cruz Biotechnologies, Inc.) monoclonal antibodies; and anti-Arp1 $\alpha$  (Sigma-Aldrich), anti-Arp2 (Santa Cruz Biotechnologies, Inc.), and anti-p34-Arc/ARPC2 (Millipore) rabbit polyclonal antibodies. For actin filament staining, we used Alexa Fluor 488–phalloidin or Alexa Fluor 546–phalloidin (Invitrogen).

### Plasmid constructs

Human  $\beta$ -actin and CH domain of human utrophin (Burkel et al., 2007) were amplified from the HeLa cDNA library by PCR and subcloned into



**Figure 5. The Arp2/3 complex is essential for the actin cluster formation and revolving movement.** (A) Arp3 siRNAs efficiently down-regulate the Arp2/3 complex. (B) Time-lapse images of HeLa cells expressing GFP-UtrCH and DsRed-histone H1. Top, control (Luc siRNA); bottom, Arp3 siRNA. Images were taken every 3 s and images are shown at 30-s intervals. Spatiotemporal representation is also shown. (C) Time-lapse images of HeLa cells expressing

pcDNA-EGFP at BamHI and EcoRI sites as described previously (Ballestrem et al., 1998). Arp3 was subcloned from pEGFP-Arp3 (Morita et al., 2007), which was a gift from K. Sobue (Osaka University, Suita, Osaka, Japan), into pcDL-SR $\alpha$ -myc or pcDNA3-HA. Silent mutations in the siRNA-targeting region of Arp3 were introduced by PCR-based mutagenesis using the following primers: forward, 5'-AGGAGTCAGCAAAAGTCGGGGAC-CAAGCTCAAAGGGAGG-3'; and reverse, 5'-CCCTCCCTTGACCTTG-GTCCCCGACCTTGACTCCT-3'.

#### RNAi

The siRNA for human Arp3 and spire, mDia1, and mDia2 were designed as described previously (Unsworth et al., 2004; Beli et al., 2008; Morel et al., 2009). As a control, the siRNA for luciferase (5'-CUUACGUGAGUA-CUUCGATT-3') was used. HeLa cells were transfected with annealed siRNA using Lipofectamine 2000 (Invitrogen), incubated overnight, washed with fresh medium, and synchronized by a double-thymidine block. The expression levels of Arp3 and mDia1 were analyzed by immunoblotting, and spire and mDia2 expression levels were analyzed by RT-PCR using the following primer sets; spire forward, 5'-CCAAGAGCGGCACTACAA-CCC-3'; spire reverse, 5'-GAGGCTCCGTGGCGTGGTGG-3'; mDia2 forward, 5'-GAAAGAATTATGAGTGAGGAG-3'; and mDia2 reverse, 5'-TCATTTCTGATGTGAACCTG-3'.

#### Cell staining, image analysis, and time-lapse observations

Synchronized HeLa cells in M phase were fixed in methanol at  $-20^{\circ}\text{C}$  for 5 min or with 3.7% formaldehyde at room temperature for 10 min. They were then permeabilized with 0.2% Triton X-100/PBS at room temperature for 10 min, washed three times with PBS, blocked with 3% BSA/PBS, and immunostained with each antibody. For Arp3 and Arp1 $\alpha$  staining, cells were preextracted in 0.5% Triton X-100 in PHEM buffer with 5  $\mu\text{M}$  taxol for 1 min, and fixed in methanol at  $-20^{\circ}\text{C}$  for 5 min. Retraction fibers were stained with Alexa Fluor 488-conjugated Phalloidin as described previously (Mitchison, 1992). Images were acquired using the DeltaVision optical sectioning system (Applied Precision) equipped with an inverted microscope (IX71; Olympus), a 60 $\times$  1.4 NA oil objective lens (Olympus), a charge-coupled device camera (CoolSNAP HQ Monochrome; Photometrics), and SoftWoRx software (Applied Precision). Image deconvolution was performed using SoftWoRx software. For live imaging, we used optical sectioning systems (DeltaVision) with a temperature-controlled and motorized stage. HeLa cells were transfected with GFP-UtrCH and DsRed-histone H1, which enabled us to monitor actin filaments and chromosome states, respectively. During the acquisition of the time-lapse images, cells were grown in the DME with 20 mM Hepes, pH 7.3, in a glass-bottomed dish (Iwaki) coated with human fibronectin (Sigma-Aldrich). Cell culture in 3D gel was performed as described previously (Toyoshima and Nishida, 2007). In brief, HeLa cells with GFP-UtrCH and DsRed-histone H1, synchronized by a double-thymidine block, were trypsinized immediately after the release from a double-thymidine block. 10,000 cells were suspended in 100 ml of Matrigel Basement Membrane Matrix (BD) and subjected to the time-lapse observations.

#### Photobleaching

We used a confocal laser microscope (FV1000-D; Olympus). HeLa cells were transfected with GFP-actin and DsRed-histone H1. After a double-thymidine block, M phase synchronized HeLa cells were observed with a 60 $\times$  oil-immersion objective lens (UPLSAPO 60 $\times$  O, NA 1.35) at wavelengths of 488 nm (GFP) and 559 nm (DsRed). Photobleaching was performed with a high-powered laser with a wavelength of 488 nm.

#### Online supplemental material

Fig. S1 shows the revolving movement of the amorphous actin cluster in HeLa cells. Fig. S2 shows the effects of the Cdk1 inhibitor Ro3306 on an M phase event. Fig. S3 shows that actin polymerization and depolymerization reactions are required for generating the mitotic actin cluster and movement. Video 1 shows the revolving movement of the amorphous actin cluster in a HeLa cell expressing GFP-UtrCH during M phase. Video 2 shows

that Cdk1 activity is required for the actin cluster formation and its revolving movement. Video 3 shows the revolving movement of the amorphous cluster of actin filaments during M phase in an MCF-7 cell expressing GFP-UtrCH and DsRed-histone H1 during M phase. Video 4 shows the revolving movement of the amorphous cluster of actin filaments during M phase in a HaCaT cell expressing GFP-UtrCH and DsRed-histone H1 during M phase. Video 5 shows the revolving movement of the amorphous cluster of actin filaments in a HeLa cell expressing GFP-UtrCH and DsRed-histone H1 under the 3D conditions during M phase. Video 6 shows the actin turnover in the amorphous cluster of actin filaments in HeLa cells expressing GFP-actin during M phase. Online supplemental material is available at <http://www.jcb.org/cgi/content/full/jcb.201007136/DC1>.

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GFP-UtrCH and DsRed-histone H1. Top, control (Luc siRNA); middle, Arp3 siRNA1; bottom, Arp3 siRNA1 + myc-Arp3res. Arrowheads indicate the actin cluster. (D) Percentages of cells showing the revolving movement of the actin cluster. Control (Luc siRNA), 100% ( $n = 22$ ); Arp3 siRNA1, 26.3% ( $n = 19$ ); Arp3 siRNA1 + myc-Arp3res, 73.3% ( $n = 15$ ); Arp3 siRNA1 + HA-Arp3res, 70.6% ( $n = 17$ ). (E) Staining with phalloidin of HeLa cells transfected with luciferase siRNA (left), Arp3 siRNA1 (middle), and Arp3 siRNA2 (right). The bottom sections show the retraction fibers, and the middle sections show the cortical actin structures and the actin cluster, which is indicated by a white circle with a yellow arrowhead. (F) Double staining with anti-Arp3 (green) and anti-Arp1 $\alpha$  (red) antibodies of HeLa cells transfected with luciferase siRNA (left) and Arp3 siRNA1 (right). Arrowheads, the patchy staining of endogenous Arp3. (G) Staining with anti-Arp3 antibody (red) of HeLa cells expressing GFP-UtrCH. Arrowheads, the actin cluster. Bars, 10  $\mu\text{m}$ .

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