A novel mechanism of intracellular transport: sieving by an anchored homogeneously contracting F-actin meshwork

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A novel mechanism of intracellular transport: sieving by an anchored homogeneously contracting F-actin meshwork

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Running title: \textbf{Transport by an anchored contractile F-actin meshwork}
Highlights

- Chromosomes in starfish oocytes are transported by an F-actin meshwork undergoing homogeneous and isotropic internal contraction;
- The direction of transport is determined by cortical anchoring of the meshwork, not by intrinsic directionality of the meshwork contraction;
- Inert beads of similar dimensions to chromosomes are transported effectively by the meshwork, suggesting transport by steric trapping (‘sieving’);
- Anchored contractile F-actin meshworks may represent a new class of versatile intracellular ‘transport machines’.
Summary

Actin-based contractility orchestrates changes in cell shape underlying cellular functions ranging from cell division to migration and wound healing, with detailed molecular mechanisms increasingly being revealed [1-5]. In addition, actin functions in intracellular transport, with the prevailing view that filamentous actin (F-actin) cables serve as tracks for motor driven transport of cargo [1, 6]. Recently, we discovered that chromosomes are transported by a contractile F-actin meshwork in starfish oocytes [7]. However, how this meshwork contracts and how its contractile activity is translated into directional transport of chromosomes remained open questions. Here, by live-cell imaging and quantitative analysis of chromosome trajectories and meshwork velocities, we show that the three-dimensional F-actin meshwork contracts homogeneously and isotropically throughout the nuclear space. Centrifugation experiments demonstrate that this homogeneous contractile activity is translated into asymmetric, directional contraction via mechanical anchoring of the meshwork to the cell cortex. Finally, this directional contraction is transduced to chromosomal cargo at least in part by steric trapping (‘sieving’), because the meshwork transports inert beads in a size-selective manner. Taken together, our results reveal mechanistic design principles of a novel and potentially versatile mode of intracellular transport based on sieving by an anchored homogeneously contracting F-actin meshwork.
Results and discussion

Oocytes have an exceptionally large nucleus, also referred to as the germinal vesicle, that stores nuclear proteins for early embryonic divisions [8]. As a consequence, specialized transport mechanisms are required to deliver chromosomes that are initially distributed throughout the nuclear space to the assembling meiotic spindle. We recently showed in starfish oocytes that an actin-dependent process transports chromosomes, initially scattered in the 80 μm-diameter nucleus, to within the capture range of centrosomal microtubule asters at the cell cortex (or animal pole, AP; Figure 1A,B), and that this process is essential to prevent chromosome loss and aneuploidy of the egg [7]. We observed that an extensive meshwork of actin filaments forms in the nuclear region and decreases in volume towards the AP during chromosome congression, and consequently suggested that this contractile meshwork drives chromosome transport [7]. However, our data at that time did not allow us to propose a specific mechanism by which the meshwork drives transport. In particular, the organization and spatial distribution of contractile activity within the F-actin meshwork, the origin of the asymmetric, directional contraction, and the mechanism by which this contraction is transduced to chromosomal cargo remained important open questions.

Chromosome trajectories suggest transport by a homogeneously contracting meshwork

To resolve the mechanism of chromosome transport by the F-actin meshwork, we first characterized chromosome trajectories during the actin-dependent phase of chromosome congression in detail. High spatial-temporal resolution movies of
fluorescently labeled chromosomes (Figure 1C and Movie S1) enabled automatic tracking of chromosome motion in 3D (Figure 1D; for details see Experimental Procedures). Trajectories exhibited the two previously identified phases of pole-ward motion: an initial slow, actin-driven phase that begins just after NEBD followed by a faster, microtubule-driven phase that begins ~10 min after NEBD [7] (Figure 1E). Quantitative analysis of the actin-driven phase of motion confirmed and extended previous qualitative observations: chromosomes begin to move simultaneously approximately one minute after NEBD and each chromosome maintains a nearly constant pole-ward speed throughout the transport process (Figure 1E, see Figure S1 for quantification). Quantitative analysis additionally revealed a novel and unexpected property of this actin-driven transport process: the constant pole-ward speed of each chromosome depends linearly on its initial distance from the AP (Figures 1F and S1). This property rules out a number of models that have been proposed to explain contraction of the F-actin meshwork, including localized contractile activity (or other localized pulling force) at the AP [7], which would result in equal pole-ward speeds for all chromosomes (see Supplementary Material for more detailed discussion of other models and their predictions for chromosome speeds). Instead, pole-ward chromosome speeds that depend linearly on initial distance from the AP imply that contractile activity is distributed homogeneously throughout the F-actin meshwork (Figure 1H).

An important corollary prediction of homogeneous contraction is that any two points in the meshwork (e.g., any pair of chromosomes) should similarly exhibit a constant relative speed of travel towards each other during the congression process, and this speed should depend linearly on their initial distance from each other. Analysis of pair-wise chromosome approach velocities confirmed this prediction (Figure 1G),
demonstrating that contractile activity is homogeneously distributed throughout the meshwork. Further, the fact that this is true for all pairs of chromosomes irrespective of their initial location in the nuclear space implies that the contraction is isotropic: it does not have an intrinsic, preferred directionality. The linear dependence of relative speed on initial separation distance was observed in more than 20 oocytes from different animals (correlation coefficient >0.8, p<<0.01), providing strong support for the homogeneous contraction model. The slope of the dependence varies from 0.3 to 1.1 min\(^{-1}\) (0.067±0.025 min\(^{-1}\) on average), suggesting that the magnitude of this homogeneous contractile activity can vary between individual cells and animals.

**Dynamics of the F-actin meshwork confirm the homogeneous contraction model**

To determine directly whether the F-actin meshwork indeed contracts homogeneously as suggested by chromosome trajectories, we imaged F-actin in live oocytes using the utrophin calponin homology domain (UtrCH [9], see Supplemental Text for details). This marker allowed us to visualize fine F-actin structures in the nuclear space with sufficient spatial and temporal resolution for quantitative analysis of F-actin bundle dynamics throughout the entire transport process (Figure 2A, Movie S2). Kymograph analysis shows that F-actin bundles form throughout the nuclear space from 0-2 min after NEBD and subsequently begin to ‘flow’ in a directed manner toward the AP (Figure 2B). As the meshwork flows toward the AP, it is continuously replenished by bundles originating at nuclear envelope (NE) remnants (Figure 2B). As a result, the entire nuclear space remains filled with the F-actin meshwork. The spatial segregation between the first contracting F-actin bundles and bundles newly produced at the NE boundary can be clearly visualized by pulse labeling the first actin filaments with
fluorescent phalloidin injected directly to the nuclear space immediately after NEBD (Figure 2C).

Manual tracing of meshwork nodes in kymographs demonstrates that F-actin structures maintain approximately constant speeds towards the AP and that these speeds are linearly dependent on the initial distance of each node from the AP (Figure 2D). In addition, automated image-correlation spectroscopy (ICS) to calculate local F-actin flows demonstrates that the velocity profile through the F-actin meshwork also increases linearly with distance from the AP (Figure 2E). These observations are consistent with the homogeneous and isotropic meshwork contraction model, and the slope of the velocity-distance dependence measured for the F-actin meshwork is indistinguishable from values obtained from chromosome trajectories (0.063 min\(^{-1}\) and 0.069 min\(^{-1}\), respectively for kymographs and ICS, compared to 0.067±0.025 min\(^{-1}\) for chromosomes).

The fact that the meshwork fills the nuclear space throughout the contraction process implies that the rates of meshwork contraction and new filament production at the membrane boundary must be balanced. To investigate the relationship between these processes, we used Latrunculin B to acutely block new filament polymerization by sequestering actin monomers. Approximately 1 min after Latrunculin B addition, new filament production at the membrane boundary arrested, coinciding with accelerated meshwork contraction (Figure 2F, Movie S3) with velocities up to ten-fold higher than in control oocytes. This observation indicates that the contractile force of the meshwork could drive much higher-speed contraction than normally observed, and that the contractile force is normally opposed by the new filaments produced at the membrane boundary. In support of this model, the membrane boundary recoils as soon as new filament production is stopped (Figure 2F, inset, Movie S3). These data
demonstrate that the net rate of contraction of the meshwork is limited by tethering to the NE remnants through new filaments produced at the membrane boundary.

**Directional contraction is achieved by meshwork anchoring**

Next, we asked how homogeneous, isotropic contraction of the F-actin meshwork is converted into asymmetric, directional contraction towards the AP. The center of mass of a homogeneously contracting meshwork will move in a directional manner if one side of the meshwork is attached to a fixed point (Figure 1H). This model assumes that contraction is independent of anchoring; thus, removing the anchor should remove only the asymmetry of contraction, not the contraction itself. Thus, we hypothesized that mechanical anchoring of the F-actin meshwork to cortical F-actin would be sufficient to render the contraction asymmetric. We tested this prediction by centrifuging oocytes [10] in order to relocate the nucleus away from the cortex and thereby remove any cortical anchoring. As a control, centrifugation of the nucleus further towards the AP did not affect chromosome transport to the cortex, ruling out adverse effects of centrifugation (Figure 3A, top panel). In support of our cortical anchoring model, relocating the nucleus to the center of the oocyte resulted in symmetric transport of chromosomes to the center of the nuclear region, indicating that meshwork contraction still occurred but had lost its asymmetric directionality (Figure 3A, middle panel). Interestingly, centrifugation of the nucleus to within ~5 μm of the cortex opposite the AP restored directionality: chromosomes were transported towards the nearest point on the cortex (Figure 3A, lower panel). Importantly, in all cases pair-wise chromosome approach velocities were linearly dependent on their initial separation distances, with slopes that are indistinguishable from control oocytes (Figure 3A), demonstrating that homogeneous internal
contraction of the F-actin meshwork occurred normally in all three conditions. Taken together, these results support our model in which mechanical anchoring of a homogeneously contracting F-actin meshwork to the cell cortex is used to drive asymmetric, directed transport in the oocyte.

These data also demonstrate that anchoring only requires close proximity of the nucleus to the cortex: it is not specific to either the AP or the position of the centrosomes and is independent of microtubules (Figure S3). A simple explanation for these observations is that the anchor consists of actin filaments that physically connect the nuclear meshwork to the cortical F-actin meshwork. This hypothesis is supported by the fact that anchoring is affected by Latrunculin B: in Latrunculin B treated oocytes, accelerated meshwork collapse coincides with a loss of directional contraction (Figure 2F), suggesting that dynamic actin filaments are required for anchoring. High resolution imaging of F-actin at the AP revealed F-actin bundles directly connecting the meshwork to the cell cortex (Figure 3B), in strong support of the hypothesis that these actin filaments form the anchor and provide the directionality of contraction.

**Steric trapping is sufficient to drive transport**

A remaining unanswered question is how the contracting meshwork transduces its motion to chromosomes in order to transport them to the AP. We previously observed that chromosomes develop dense F-actin structures in their vicinity that could potentially serve to attach them to the meshwork via specific binding interactions ([7], Figure S2). However, our new high resolution data revealed that these dense structures are specific to chromosomes located near the NE, and many chromosomes scattered in the nuclear space are transported in the absence of any visible specific F-
actin structure (Figure 4A, Movie S4). This suggested that chromosomes may be transported without direct binding to F-actin, simply by steric trapping within the meshwork. To directly test whether transport can occur in the absence of specific binding interactions, we injected a dense polydisperse mixture of inert fluorescent beads and bead aggregates into the oocyte nucleus and imaged them in 3D during chromosome congression. We found that the effective volume occupied by these inert particles decreased over time towards the AP, indicating that they were also transported by the actin meshwork. The extent of the volume decrease was size-dependent: we found that the effective radius of the space occupied by three different bead size groups decreased linearly over time at a rate that increased with particle size (Figure 4B). Importantly, the space occupied by the largest particle group (with an estimated average diameter of 0.7 μm) decreased with a rate approaching that of chromosomes (~2 μm diameter) imaged in the same cell (Figure 4B). To analyze the transport of these large particles in detail, we injected oocytes with large bead aggregates at sufficiently low density to track them over time (Figure 4C). Their trajectories revealed behavior strikingly similar to that of transported chromosomes: bead aggregates synchronously initiate directed pole-ward movement shortly after NEBD; their pole-ward speeds appear constant during transport; and their pole-ward and relative pair-wise approach speeds are linearly dependent on initial separation distance, with a slope that is indistinguishable from that of chromosomes within measurement error (0.077 min⁻¹ compared to 0.067±0.025 min⁻¹, Figure 3D,E).

These results demonstrate that the F-actin meshwork can effectively mediate transport without specific binding interactions with the cargo. The fact that transport efficiency depends on particle size implies that the force of meshwork contraction is transmitted to cargo at least in part by steric trapping within porous ‘cages’ in the meshwork:
smaller particles are more likely than large particles to escape through gaps between actin bundles. The bead experiments indicate that efficient capture requires particles of roughly 1 μm diameter, defining an approximate effective mesh-size of the meshwork. Particles larger than this size are transported with an efficiency and rate similar to that of chromosomes. Importantly, this apparent mesh size for transport is in good agreement with the mesh size visible in high resolution images of actin bundles that constitute the meshwork (Figure 4F,G). Thus, these visible bundles are likely the structures mediating particle trapping and transport.

**Anchored contractile F-actin meshworks as a new class of intracellular ‘transport machines’**

Detailed quantitative analyses of F-actin-driven chromosome transport together with specific experimental perturbations in starfish oocytes allowed us to identify the design principles of a novel and potentially versatile intracellular ‘transport machine’ (Figure 4H) that is fundamentally distinct from previously observed mechanisms of F-actin driven intracellular transport. In this system, force is generated by isotropic contractile activity that is distributed homogeneously throughout the F-actin meshwork, with a rate of contraction that is limited through tethering by filament production at the NE remnant membranes. Mechanical anchoring of the meshwork to the cell cortex by actin filaments confers directionality to the contraction. Finally, the contractile force is conveyed to cargo at least in part by steric entrapment of particles larger than the effective mesh-size of the meshwork.

In starfish oocytes, this mechanism is utilized for the essential function of transporting chromosomes to the AP. However, it is tempting to speculate that similar design principles may be used in other intracellular transport processes in various organisms.
and cell types. This transport system is inherently flexible in that anchoring to cellular structures other than the cortex may direct contraction to distinct subcellular locations, and tuning the effective mesh-size could allow selective transport based on size. Alternatively, specific binding interactions between cargo and the meshwork could potentially enhance the rate and reliability of transport. Notably, in contrast to chromosome transport by microtubules, where re-establishment of severed microtubule-chromosome connections requires significant time and may result in chromosome loss [11], transport by a space-filling F-actin meshwork may be considerably more robust due to physical entrapment within an extended meshwork that allows for rapid recapture.

Elucidating the detailed molecular mechanisms underlying each of the functional components that we have identified in this novel intracellular transport machine is an important goal of future work. In particular, our finding that contractile activity is homogeneously distributed throughout the actin meshwork suggests that the meshwork may be organized into quasi-independent contractile F-actin subunits, as recently also proposed for the contractile ring of *C. elegans* embryos [12] and stress fibers in cultured mammalian cells [13]. However, unlike those one-dimensional actomyosin systems, the actin meshwork here consists of a complex three-dimensional arrangement of discrete bundles, so that meshwork structure and connectivity must be considered in addition to the molecular composition of individual bundles. We showed previously that depolymerization is required for contraction [7], which is consistent with our present data (Figure 2F and S2C), but whether depolymerization drives contraction remains unknown. Our attempts to test the involvement of myosins remained inconclusive ([7], data not shown). Therefore, how individual bundles forming the meshwork are organized to generate contractile
force, and whether this force is generated by actomyosin contractility, depolymerization/bundling of actin filaments independent of motor activity [12, 14-16], or some combination thereof pose challenging questions to address in future work. Nevertheless, it is an intriguing possibility that contractile units with similar structure and composition to those that form flat networks under the cell membrane to mediate cytokinesis or cell migration may alternatively organize into 3D F-actin meshworks to drive intracellular transport.

**Supplemental Data**

Supplemental Data include Supplemental Text, Experimental Procedures, three figures, and four movies and can be found with this article online.
Acknowledgements

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References

Figure legends

Figure 1. Chromosome trajectories suggest transport by homogeneous contraction

(A) Schematic of an immature starfish oocyte, showing the nucleus anchored at the animal pole (AP) and chromosomes scattered throughout the nuclear volume.

(B) Schematic of the process of actin-driven chromosome congression that begins after nuclear envelope breakdown (NEBD), and during which the nuclear space occupied by chromosomes shrinks to approximately one-half of its original diameter. Dashed lines indicate the position of the nuclear envelope (NE) prior to NEBD.

(C) Pseudo-colored time projection of all maximum intensity z-projections through the nuclear region of an oocyte expressing H2B-mCherry. 27 z-sections were acquired every 15 s. H2B-mCherry also labels the nucleolus (n) that disassembles after NEBD. Time is relative to NEBD, scale bar: 10 μm. For the full movie see Movie S1.

(D) Trajectories of chromosomes automatically tracked from the 3D dataset shown in (C). Trajectories are colored during the actin-driven transport phase (the color of each trajectory is consistent throughout Figures 1D-F and S1).

(E) Trajectories in (D) plotted as distance from the AP versus time. (1) Start of slow actin-driven transport; (2) start of capture by microtubules. Shaded and colored portions of the trajectories (from 2–8 min after NEBD) are used in subsequent analyses of the actin-driven transport phase.

(F) Dependence of pole-ward chromosome speeds on initial chromosome distance from the AP. Speeds were calculated as described in Experimental Procedures and Figure S1. C_{corr}: correlation coefficient.
(G) Dependence of pair-wise chromosome approach speeds on the initial distance between each pair of chromosomes, calculated as described in Experimental Procedures. \( C_{\text{corr}} \): correlation coefficient.

(H) Schematic of the homogeneous contraction model, illustrating the dependence of speed towards a fixed point (anchor) on distance from the anchor. Homogeneously distributed contractile activity is represented as contractile elements (lines between nodes). The regular arrangement of the contractile elements in the 2D model is only for visualization purposes and is not a specific feature of the model.

**Figure 2. F-actin dynamics confirm homogeneous meshwork contraction**

(A) Selected single confocal sections through the nuclear region of an oocyte expressing UtrCH-3mEGFP (to label F-actin; gray) and injected with DiIC\(_{18}(3)\) (to label endomembranes; red). The F-actin meshwork forms in the nuclear space defined by the remnant NE membranes. Time is shown relative to NEBD, (n) labels the disassembling nucleolus, which excludes F-actin. For the complete dataset see Movie S2.

(B) Kymograph along the animal-vegetal axis as indicated in (A) in an oocyte expressing the same markers as in (A). Arrowheads mark some of the new actin structures produced at the membrane boundary.

(C) Rhodamine-phalloidin was injected into the nuclear space \( \sim 2 \) min after NEBD in an oocyte expressing UtrCH-3mEGFP to pulse label the population of filaments present at the time of injection. A single selected frame \( \sim 2 \) min after injection is shown from a time series.

(D) Left: traced F-actin structures overlaid on a kymograph from an experiment similar to (B). Right: pole-ward meshwork structure velocities calculated from lines
fit to the traces versus the initial distance of each structure from the AP. Initial distances were extrapolated to the start of the actin-driven transport phase using the fitted velocities. Colors are consistent between panels.

(E) Image correlation spectroscopy (ICS) analysis performed using the dataset shown in (D), using a one-minute time interval during the middle of actin-driven transport (4-5 min after NEBD) and a sliding 50x50 pixel (7.5x7.5 μm) template. Left: white arrows show measured flow velocities overlaid on a selected frame; right: pole-ward component of the measured velocities plotted as a function of distance from the AP. The slope of the linear correlation, extrapolated to the start of the actin-driven transport phase, is shown for comparison with the kymograph tracing in (D).

(F) Selected frames and kymographs of oocytes labeled and imaged as in (A) and (B), respectively. The oocyte to the left was treated with DMSO to serve as control, while the oocyte to the right was treated with 10 μM Latrunculin B at the time marked by a gray arrowhead. Dashed lines mark the y-positions shown in the kymographs. Approximately 1 min after Latrunculin B treatment the membrane boundary recoils (white arrowhead); this is most apparent at y-positions where a filament bundle was directly attached to the membrane (inset). Time is relative to NEBD. For the complete datasets see Movie S3. All scale bars are 20 μm.

**Figure 3. Directionality is achieved by anchoring the meshwork to the cell cortex**

(A) Oocytes expressing H2B-GFP and injected with rhodamine-tubulin for identification of the AP were centrifuged in order to relocate the nucleus either back towards the AP as a control (top row), to the center of the cell (middle row), or to the opposite cortex (bottom row). Left-most column: low magnification images of rhodamine-tubulin before NEBD. The smaller dark circle in the left middle image is
the negative image of the oil drop used for injection. Middle columns: maximum intensity z-projections of the nuclear region, marked by a dashed rectangle in the left-most column, showing H2B-GFP labeled chromosomes at NEBD and at the end of chromosome transport, and pseudo-colored time projections of z-projections during chromosome transport; dashed ellipses label the initial position of the NE. Right-most columns: pair-wise chromosome approach velocities versus initial pair-wise separation distance, calculated as in Figure 1G, for the chromosome trajectories obtained from each of the three datasets.

(B) Selected frame from a high resolution time series of the AP in an oocyte expressing UtrCH-3mEGFP (top). A filament connecting the nuclear region meshwork to the cell cortex (red arrowheads) is visible in an inset from the selected frame (middle), and a subsequent frame (bottom) shows a different filament coming into focus. Scale bars: 10 μm.

Figure 4. Sieving can mediate transport

(A) Selected frame from a time series along the animal-vegetal axis of an oocyte injected with recombinant UtrCH-Alexa488 (gray) and H1-Alexa568 (red). The kymograph is generated from the region marked with a dashed rectangle. Scale bar: 10 μm. For the complete movie see Movie S4.

(B) Upper left: maximum intensity projections of 20 z-sections showing single and aggregated 0.4-μm diameter PEG-coated fluorescent beads injected into the nucleus of an oocyte. Time is given relative to NEBD. Lower left: particles identified and color-coded based on size categories (defined based on their relative intensities) and the effective radius of the occupied volume for each category (calculated as the radius of gyration of each set of particles). Right: the radius of the occupied volume of the
set of particles in each size group is plotted over time (each point is an average over five time frames). The gray line shows the same analysis performed on chromosomes that were labeled by H1-Alexa647 in the same experiment. Scale bar: 20 μm.

(C) Pseudo-colored time projection of z-projections of an oocyte injected with aggregates of 0.5 μm diameter PEG-coated fluorescent beads. Scale bar: 20 μm.

(D) Distance from the AP versus time for the trajectories obtained from the dataset shown in (C). The actin-driven phase is highlighted; colors are consistent with (E). See Figure 1E for comparison with chromosomes.

(E) Pole-ward (colored filled circles) and pair-wise (+) velocities (calculated as for Figure 1F,G) versus initial distance from the AP and initial pair-wise distance, respectively.

(F) F-actin meshwork labeled by UtrCH-3mEGFP in a live oocyte, single confocal section, scale bar: 10 μm. On the inset red dots mark imaginary 1 μm diameter particles for comparison of scales.

(G) F-actin meshwork labeled in a fixed oocyte by Alexa488-phalloidin, single confocal section, scale bar: 10 μm. On the inset red dots mark imaginary 1 μm diameter particles for comparison of scales.

(H) Schematic of the homogeneous contraction model for chromosome transport. See text for details.
Figure 1

A Immature oocyte

Animal pole (AP)

Vegetal pole (VP)

~180 μm

n

B NEBD

NEBD + 10 min

AP

nuclear space

~80 μm

Actin-driven chromosome congression ~10 min

~40 μm

C Time projection of raw data

D Chromosome tracks

E Distance from the AP versus time

F Pole-ward speeds

G Pair-wise speeds

H Homogeneous contraction model

1D model

2D model
Figure 2

A Overview of F-actin meshwork dynamics
UtrCH-3mEGFP / DiIC$_{18}$(3)

B Kymograph along the AV axis
UtrCH-3mEGFP / DiIC$_{18}$(3)

C Pulse labeling by Rh-phalloidin
Rh-phalloidin UtrCH-3mEGFP Merge

D Kymograph tracing
UtrCH-3mEGFP

E ICS analysis
Local F-actin speed vs. distance

F Control 3 min Latrunculin B 3 min Latrunculin B addition

Meshwork speed vs. initial distance
Slope: 0.063±0.005 min$^{-1}$
$C_{corr} = 0.75; R^2 = 0.56$

Speed towards the AP (μm/min) 0 1 2 3 4 5
Distance from AP (μm) 0 10 20 30 40 50
Slope at start: 0.069±0.001 min$^{-1}$
Figure 3

A Relocation of the nucleus by centrifugation

Nucleus centrifuged to the AP (control)

Rho-amine-tubulin before NEBD
H2B-GFP NEBD
End of congression

Time projection 0-10 min after NEBD

Pair-wise speeds

Nucleus centrifuged away from the cortex

Nucleus centrifuged to the cortex opposite the AP

B Anchoring filaments

Figure 3

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Figure 4

A Chromosome transport by the F-actin meshwork

UtrCH-A488 / H1-A568

B Size dependent transport of beads

Z-projection

C Time projection

D Pole-ward approach

E Distance dependence of speed

F

G

H Model of actin-driven chromosome transport
Inventory of Supplemental Information

1. Supplemental Material

MS-Word file containing Supplemental Text, Experimental Procedures and Supplemental Figure and Movie legends.

2. Supplemental Figure 1

A pdf file of Figure S1, related to Figure 1.

3. Supplemental Figure 2

A pdf file of Figure S2, related to Figure 2.

4. Supplemental Figure 3

A pdf file of Figure S3, related to Figure 3.

5. Supplemental Movie 1

An MPEG-4 QuickTime .mov file related to Figure 1.

6. Supplemental Movie 2

An MPEG-4 QuickTime .mov file related to Figure 2.

7. Supplemental Movie 3

An MPEG-4 QuickTime .mov file related to Figure 2.

8. Supplemental Movie 4

An MPEG-4 QuickTime .mov file related to Figure 4.
Competing models of chromosome transport

The high degree of coordination observed between chromosomes was difficult to reconcile with prevailing views of actin-dependent intracellular transport—transport by myosin motors along F-actin cables or propulsion by F-actin ‘comet tails’ polymerized at the surface of the transported object—therefore we excluded these mechanisms from consideration and focused on models in which a nuclear-level, interconnected structure mediates transport. Three spatially distinct components of the meshwork (Figures 2A and S2A,B) must be considered as playing potential functional roles in transport: (1) the meshwork boundary at the NE remnants; (2) the animal pole (AP), towards which transport is directed, where the centrosomes are located and where the meshwork is proximal to the cell cortex; and (3) the interior of the nuclear space that is filled by the meshwork. We considered three competing models in which these components play distinct roles in the transport process (Figure S1D):

Competing Model 1: “Fishnet”

In this model, the outer boundary of the meshwork (which forms initially at NEBD; see description of the actin ‘shell’ below) interacts with and conveys force to chromosomes, transporting them like a peripheral ‘fishnet’ that is reeled in to the AP. The reeling process could be driven by contractile activity either at the AP or throughout the meshwork boundary. In either case, this model predicts that chromosomes initiate directed motion sequentially as the constricting boundary reaches them on its way to the cortex. This prediction is clearly contradicted by our observation that chromosomes initiate directed motion at the same time, independent of their initial position within the nuclear region (Figure S1A).
Competing Model 2: “Active pole”

In this model, the full three-dimensional meshwork interacts with and conveys force to chromosomes but contractile activity is localized at the AP; in other words, transport is driven by a passive meshwork that is reeled in at the AP, as opposed to active contraction that is distributed homogeneously throughout the meshwork. This model predicts that all chromosome speeds towards the AP are equal, set by the pulling speed at the AP. This model is ruled out because chromosomes do not move with equal speeds (Figure S1C).

Working Model: “Anchored homogeneously contracting meshwork”

In this model, in contrast to Competing Model 2, the AP plays a passive, anchoring role that provides directionality to homogeneous and isotropic contraction distributed throughout the meshwork. This model predicts that pole-ward chromosome speeds depend linearly on initial distance from the AP. This prediction results from the fact that the relative speed between any two points in a homogenously contracting meshwork is proportional to the effective number of contractile subunits connecting them, which is in turn proportional to their initial separation distance (Figure 1H). Our analysis of chromosome speeds confirms this property (Figure S1C), supporting the anchored homogeneous contraction model as the mechanism of chromosome transport.

F-actin structures in the nuclear space and their potential functions in chromosome transport

Here we employed UtrCH-3mEGFP [1] to label F-actin in live oocytes. Due to its high affinity for F-actin and low soluble background, this marker enabled us to image F-actin dynamics in live oocytes with approximately 5-fold higher temporal and
significantly improved spatial resolution. To exclude the possibility that this maker alters F-actin structures or only labels a subset of filaments, we carefully compared the results obtained with UtrCH to other markers including ABD-120 [2] and ITPKA ([3], data not shown), as well as to oocytes fixed and stained with rhodamine-phalloidin (Figure S2B). All methods gave consistent results and revealed three distinct F-actin structures present during the chromosome transport process (Figure S2A): (1) an initial shell of F-actin that forms at NEBD along the breaking NE; (2) dense ‘patches’ of F-actin that surround chromosomes; and (3) a meshwork of F-actin bundles/filaments that fills the entire nuclear space.

The F-actin shell persists only for ~1.5 min after NEBD and disassembles as chromosomes are just beginning to move. Therefore, the F-actin shell is unlikely to play a direct active role in chromosome transport. Our high resolution UtrCH data also unexpectedly revealed that F-actin patches only form around chromosomes close to the nuclear periphery, while chromosomes deeper within the nuclear space do not accumulate visible F-actin (Figure S2A,B and 4A). This implies that F-actin patches are unlikely to be essential for chromosome transport, since some chromosomes are transported without a detectable patch. Our analyses of chromosome trajectories did not reveal distinct subpopulations of chromosome behaviors that would be expected if chromosomes with and without an F-actin patch were transported by different mechanisms (Figure S1C). Nevertheless, F-actin patches may have an important function in securing peripheral chromosomes to the meshwork and preventing chromosome loss.

These observations demonstrate that neither the initial F-actin shell nor the F-actin patches around chromosomes can mediate the transport of all chromosomes through the 10 min congression process. In contrast, the F-actin meshwork fills the entire
nuclear space and persists throughout chromosome congression. Therefore, the rest of this manuscript focuses on the F-actin meshwork.

**Experimental procedures**

**Oocyte injection, maturation and centrifugation**

Starfish (*Patiria miniata*, a.k.a. *Asterina miniata*) were obtained from Southern California Sea Urchin Co. (Corona del Mar, CA) and maintained in sea water tanks at 16 °C at EMBL’s Marine Facility. Oocytes were isolated and injected using mercury-filled needles as previously described [4] (see also http://mterasaki.us/panda/injection/). Recombinant protein markers were injected shortly (0.5-1 h) before initiation of meiosis, whereas mRNA injections were done the day before and incubated overnight at 14 °C to obtain sufficient levels of protein expression. Meiotic maturation was triggered by the addition of 10 μM 1-methyladenine (Sigma). NEBD typically started 20 min after hormone addition, and only oocytes starting NEBD between 15 and 35 min were analyzed. Centrifugations were done principally as described in [5] with small modifications. Briefly, oocytes were placed into coverslip shelves used for microinjection and these coverslips were then positioned into a plastic holder in a sea water-filled 50 ml conical tube; this assembly was centrifuged at 900 G at 4 °C for 50 min.

**Fluorescent markers and microscopy**

H2B-GFP, H2B-mCherry [6] and UtrCH-3mEGFP [1] were subcloned into pGEMHE for *in vitro* transcription as described in [4]. Capped mRNAs were synthesized from linearized templates using the mMessage mMachine kit (Ambion), dissolved in 11 μl
RNase-free water (typically at 1-2 μg/μl) and injected to 1-5% of the oocyte volume. Rhodamine-tubulin was purchased from Cytoskeleton Inc. (Denver, CO), dissolved as recommended by the supplier, dialyzed to remove residual glycerol and injected to oocytes. Histone H1 from calf thymus (Merck) was dissolved in a buffer containing 0.5 M NaCl and 50 mM HEPES at pH 8.0 and labeled with Alexa Fluor 568 succinimidyl ester (Invitrogen) according to the manufacturer’s instructions, purified by gel filtration (PD-10, GE Healthcare) and concentrated by Vivaspin columns (10,000 MW, Sartorius) before injection to oocytes. UtrCH [1] was subcloned into pET24d(+) for expression in E. coli. The recombinant protein was expressed for 1 h at 37 °C and purified on a Ni-NTA agarose resin (QIAGEN). The purified protein was labeled by Alexa Fluor 488 succinimidyl ester (Invitrogen) according to the manufacturer’s instructions, purified and concentrated as above. Streptavidin-coated 0.4 and 0.5 μm diameter green fluorescent microspheres (Bangs Labs Inc.) were incubated with biotin-PEG-OH (MW 3,000) (Iris Biotech GmbH) and 3% BSA in PBS overnight at RT to make them inert and electrostatically neutral [7]. It was technically not possible to inject larger beads into nuclei, therefore we incubated beads for different times at 4 °C to allow for formation of aggregates, and injected this mixture into oocyte nuclei. DiIC$_{18}$(3) (Invitrogen) was dissolved in vegetable oil to saturation and injected into oocytes as in [4]. The methanol stock of rhodamine-phalloidin (Invitrogen) was dried and dissolved in water and amounts corresponding to $10^{-5}$ units were injected directly into the nuclear region of oocytes.

Microscopy was done either on a customized Zeiss LSM510, LSM510 Meta or LSM710 Axiovert confocal microscope equipped with a fast z-focusing device (HRZ or objective piezo) and using a 40x C-Apochromat 1.2 NA water immersion objective lens (all Zeiss). For drug treatments oocytes were treated directly in the injection
chambers or transferred into plastic dishes (Ibidi #80131). Nocodazole (Calbiochem) was used at 3.3 μM and in acute treatments Latrunculin B (Invitrogen) was added at 10 μM final concentration.

**Image processing and particle tracking**

Image processing and data analysis was done using ImageJ (http://rsb.info.nih.gov/ij) and Matlab (MathWorks Inc.). Zeiss .lsm files were imported by the ImageJ LSMToolbox plugin (by Patrick Pirrotte and Jerome Mutterer, IBMP, Strasbourg) or by the Matlab function tiffread (by Francois Nedelec, EMBL); kymographs were traced using NeuronJ [8]; and time projections were done using the TimeRGBcolorcode macro by Kota Miura (EMBL).

Chromosome tracking and data analysis were done by in-house routines written in Matlab. Our segmentation and tracking algorithm will be described in detail elsewhere (Monnier et al., manuscript in preparation). Briefly, chromosomes were segmented from Gaussian-filtered and background-subtracted 3D image stacks at each time point by thresholding. The optimal threshold level was determined automatically by identifying a peak in the mean volume of features detected as a function of threshold level. After segmentation at each timepoint, detected objects were assigned to tracks based on the distance between each object and both the expected position and last position of each track. Objects to which multiple tracks were assigned (i.e. merges) were split between the tracks by intensity-based watershedding or by partitioning their total intensity between the tracks in proportion to the relative intensity of the tracks in the previous timepoint. The resulting tracks for each movie were manually screened for accuracy before being included in subsequent analysis.
The directionality of pole-ward transport in Figure S1A was defined as the cosine of the angle between the direction towards the AP and the direction of the principle component of motion in 3 min (12 time steps) time windows along the tracks [9]. A threshold was set at an angle of 30 degrees from the pole-ward direction to define the start time of directed motion. Chromosome speeds during the actin-driven transport phase (the time interval from 2-8 min after NEBD) were determined by three different methods: fitting a line to the distance from the AP versus time (i.e. finding the linear pole-ward component of the velocity), fitting a line to the trajectory in 3D space, or fitting a diffusion-flow model to the mean-squared displacement along the trajectory (see Figure S1B). As all three methods gave consistent results, we employed the first method in the main figures. Pair-wise relative chromosome velocities were similarly defined as the slope of the best-fit line to the separation distance between the pair versus time. For the bead analysis, particles were segmented from the 3D dataset and split into three size groups at each time point based on their intensities (Figure 4B): the smallest 50% of particles, the 50-75% range of particles, and the upper 25% of particles; these size groups had average intensities in the ratio of 1 bead to 2 beads to 5 beads, respectively. We estimated the space occupied by these bead size groups by calculating the radius of gyration of the set of bead positions in each size category. For the flow analysis of the actin meshwork, flow velocities were calculated by the ICS method published in [10]. Throughout the figures, slopes of linear fits are given with ± standard deviations, and the $R^2$ value and the correlation coefficient are also shown. Matlab routines are available on request. Figures were assembled in Adobe Illustrator (Adobe Systems Inc.).
Supplemental references


Supplemental figure and movie legends

Figure S1. Quantitative analyses of chromosome motion (related to Figure 1)

(A) Left: the directionality of transport towards the AP, defined as described in Experimental Procedures, plotted over time for three chromosomes selected from the dataset in Figure 1C. The colors used for individual chromosomes are consistent with those in Figure 1. The horizontal dashed line marks the threshold for directional transport, set at 30 degrees from the pole-ward direction. Right: start times of all chromosomes, defined as the first time point along the track where the directionality was above the threshold, plotted with their distance from the AP at the identified start time on the y-axis. The vertical red line and the boundaries of the gray shaded region illustrate the mean and standard deviation of the start times for all chromosomes.

(B) Speeds for each chromosome during the actin-driven transport phase (2-8 min after NEBD) were determined by fitting a line to the distance from the AP versus time (i.e. the pole-ward component of the velocity) (left), fitting a line to the trajectory in 3D space (middle), or fitting a diffusion-flow model to the mean-squared displacement along the trajectory (right). Three chromosomes selected from the dataset in Figure 1 are shown as examples. The resulting speeds are shown in units of μm/min.

(C) Comparison of the chromosome speeds calculated by the three methods in (B), plotted versus initial chromosome distance from the AP. The linear fits to these three datasets (with slopes given in the legend) are identical within measurement error. Color coding corresponds to that of Figure 1F.

(D) Competing models of chromosome transport. See Supplemental Text for details.
Figure S2. F-actin structures present during F-actin-driven chromosome transport (related to Figure 2)

(A) An oocyte expressing UtrCH-3mEGFP (gray) and injected with H1-Alexa568 (red) was imaged through meiotic maturation. Maximum intensity projections of 10 z-sections through a portion of the nuclear region are shown at select time points (time relative to NEBD). (n) labels the disassembling nucleolus.

(B) An oocyte was fixed and stained with Alexa488-phalloidin (gray) and Hoechst 33342 (red). A maximum intensity projection of 20 z-sections is shown.

(C) The total amount of F-actin constituting the meshwork inside the nuclear envelope remnants at each time point was estimated from a 3D time series of oocytes expressing UtrCH-mEGFP and injected with DiIC$_{18}$(3). The nuclear volume was segmented using the DiI image to identify the nuclear envelope remnants, and the total intensity of F-actin (minus background) within this volume was normalized and plotted over time. Control (blue trace) and an oocyte treated with Latrunculin B 6 min after NEBD (red trace, Latrunculin B addition is marked by a horizontal black line) are shown. The traces reveal net depolymerization of the meshwork during the contraction process (from around 3-10 min after NEBD, after initial meshwork formation is completed). Time is relative to NEBD. Scale bars are 20 μm.

Figure S3. Cortical anchoring is independent of microtubules (related to Figure 3)
Oocytes expressing H2B-GFP were treated at different times with 3.3 μM nocodazole to depolymerize microtubules. Left: maximum intensity z-projections of the nuclear region of oocytes at five selected time points (time relative to NEBD) during chromosome transport. Middle: pseudo-colored time projections of z-projections of all time points during chromosome transport. Dashed circles label the initial position
of the NE. Scale bars: 10 μm. Right: pair-wise chromosome approach velocities calculated as for Figure 3A.

Upper row: control experiment performed in the absence of nocodazole.

Middle row: nocodazole added 4 h before NEBD, leading to detachment of the nucleus from the AP (due to the fact that centrosomal microtubules are required to hold the nucleus at the AP in immature oocytes before NEBD; see [11] and our unpublished results), with the same effect on chromosome transport as centrifuging the nucleus to the cell center (Figure 3A).

Bottom row: nocodazole added 10 min before NEBD, resulting in disruption of microtubules but insufficient time for the nucleus to detach from the AP. In this case, chromosome behavior is similar to the control experiment with the exception that microtubule capture (the second phase of motion in Figure 1E) does not occur due to the absence of microtubules.

**Movie S1. Chromosome congression in starfish oocytes** (related to Figure 1)

Maximum intensity projections of 27 z-sections through the nucleus of an oocyte expressing H2B-mCherry during chromosome congression. The same dataset is shown and analyzed in Figure 1 and S1. The first section of the movie shows the raw data, the second the filtered data (see Experimental Procedures), followed by a 3D rotation of the time projection.

**Movie S2. Overview of F-actin meshwork dynamics** (related to Figure 2)

A single confocal section through the nuclear space of an oocyte expressing UtrCH-3mEGFP (gray) and injected with DiIC$_{18}$(3) (red), imaged over time during
chromosome congression. Selected time points from this dataset are shown in Figure 2A.

**Movie S3. Latrunculin B treatment results in rapid collapse of the meshwork** (related to Figure 2)
Single confocal sections through the nuclei of oocytes expressing UtrCH-3mEGFP (gray) and injected with DiIC\(_{18}\)(3) (red), imaged over time during chromosome congression. The upper oocyte is an untreated control, the lower oocyte was treated at the indicated time with 10 \( \mu \)M Latrunculin B. Selected time points and kymographs from this dataset are shown in Figure 2F.

**Movie S4. The contractile F-actin meshwork transports chromosomes** (related to Figure 4)
A single confocal section through the nucleus of an oocyte injected with recombinant UtrCH-Alexa488 (gray) and H1-Alexa568 (red), imaged over time during chromosome congression. A selected frame is shown in Figure 4A.
Supplemental Figure 1

A Start of pole-ward directed motion
Directionality of selected chromosomes

B Chromosome speeds

C Distance dependence of chromosome speeds

D Competing models of chromosome transport
Supplemental Figure 2

A Overview of F-actin dynamics during chromosome transport

UtrCH-3mEGFP / H1-A568

B Meshwork in fixed cells

Alexa488-phalloidin / Hoechst 33342

C Estimate of total F-actin amounts

Relative total F-actin in meshwork

-0.2 0 0.2 0.4 0.6 0.8 1 1.2

-1 0 2 4 6 8 10 Time (min)

- Latrunculin B
- Control
Supplemental Figure 3

A

Control
H2B-GFP
NEBD

+3 min
+6 min
+9 min
end of congression

Time projection
0-10 min after NEBD

Pairwise speeds

Approach speed (μm/min)

Initial distance (μm)

Slope: 0.069 min⁻¹
C_corr = 0.90

Slope: 0.089 min⁻¹
C_corr = 0.81

Slope: 0.060 min⁻¹
C_corr = 0.82

Nucleus detached (nocodazole added long before NEBD)

Nucleus attached (nocodazole added just before NEBD)