Chromosome- and spindle-pole-derived signals generate an intrinsic code for spindle position and orientation

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Mitotic spindle positioning by cortical pulling forces¹ defines the cell division axis and location, which is critical for proper cell division and development³. Although recent work has identified developmental and extrinsic cues that regulate spindle orientation⁴–⁶, the contribution of intrinsic signals to spindle positioning and orientation remains unclear. Here, we demonstrate that cortical force generation in human cells is controlled by distinct spindle-pole- and chromosome-derived signals that regulate cytoplasmic dynein localization. First, dynein exhibits a dynamic asymmetric cortical localization that is negatively regulated by spindle-pole proximity, resulting in spindle oscillations to centre the spindle within the cell. We find that this signal comprises the spindle-pole-localized polo-like kinase (Plk1), which regulates dynein localization by controlling the interaction between dynein–dynactin and its upstream cortical targeting factors NuMA and LGN. Second, a chromosome-derived RanGTP gradient restricts the localization of NuMA–LGN to the lateral cell cortex to define and maintain the spindle orientation axis. RanGTP acts in part through the nuclear localization sequence of NuMA to locally alter the ability of NuMA–LGN to associate with the cell cortex in the vicinity of chromosomes. We propose that these chromosome- and spindle-pole-derived gradients generate an intrinsic code to control spindle position and orientation.

The position and orientation of the mitotic spindle are determined by forces generated at the cell cortex⁴, where astral microtubules emanating from the mitotic spindle pole are anchored to the plasma membrane⁴. To understand the intrinsic mechanisms that control spindle positioning, we first observed the mitotic cortical localization of established players that contribute to spindle orientation⁴–⁷ including the minus-end-directed microtubule-based motor cytoplasmic dynein, dynactin, NuMA and LGN, the human homologue of Caenorhabditis elegans GPR-1/2 and Drosophila Pins. In HeLa cells, LGN localizes to the cell cortex from prometaphase to telophase (Fig. 1a), whereas dynein and the dynactin subunit Arp1A accumulate at the cell cortex subsequently to LGN (Fig. 1a; data not shown). Consistent with this ordered temporal localization, we found that LGN was required for the cortical localization of dynein–dynactin (Fig. 1b and Supplementary Fig. S1a,b). LGN depletion specifically disrupts cortically localized dynein–dynactin, but not dynein localized to the kinetochore, spindle or spindle pole (Supplementary Fig. S1c). Finally, consistent with previous work⁵–⁹, we found that the LGN-binding proteins Gto1 and NuMA were required for LGN and dynein localization to the cell cortex (Fig. 1b and Supplementary Fig. S1d–f).

Live-cell imaging revealed that LGN showed a symmetric distribution to the cell cortex during metaphase, even if the spindle is displaced from the centre of the cell (Fig. 1a). NuMA and Gto1 also exhibited symmetric cortical localization, although Gto1 showed more homogeneous localization (Supplementary Fig. S2a,c). In contrast, Arp1A accumulated asymmetrically at the cell cortex during metaphase such that it is preferentially localized to the cortex that is distal to the mitotic spindle (Fig. 1a). All investigated dynein and dynactin subunits exhibited similar asymmetric localization to the cell cortex in HeLa (Supplementary Fig. S2a,b,d,e) and non-transformed Rpe1 cells (Supplementary Fig. S2f). To analyse the effect of asymmetric cortical dynein localization on spindle movement during metaphase, we acquired time-lapse movies of HeLa cells stably expressing dynein heavy chain (DHC)–GFP to monitor both cortical dynein and spindle poles (Fig. 1c). Kymographs of these movies revealed that the spindle moves towards the dynein-enriched side of the cell and oscillates as cortical dynein is dynamically redistributed (Fig. 1c and Supplementary Fig. S3a,b). Most cells (n = 24/26) exhibited at least one complete spindle oscillation event with the spindle moving at ~0.4 μm min⁻¹, but decreasing as the oscillations dampen and the spindle becomes aligned (Supplementary Fig. S3b). Importantly, we found that LGN depletion eliminates spindle oscillations as well as cortical dynein localization (n = 21; Fig. 1c), indicating that cortical dynein may be responsible for...
Dynein and dynactin localize asymmetrically to the cell cortex during metaphase. (a) Left, time-lapse images from a clonal HeLa cell line stably expressing GFP–LGN and mCherry–Arp1A. LGN localizes to the cell cortex before Arp1A, and exhibits symmetric localization. Arp1A exhibits asymmetric localization to the cell cortex when the spindle is mis-positioned (arrowhead). Right, graph of relative fluorescence intensity for a line scan of the indicated lines showing the spatial distribution of LGN and Arp1A. (b) Schematic showing the dependency relationships and symmetric–asymmetric behaviour for cortical localization. (c) Kymographs showing DHC–GFP and chromosomes (Hoechst) generated from time-lapse movies at 5 min intervals as indicated illustrating the oscillation of the spindle and its effect on cortical dynein localization in MG132-arrested control cells, LGN-depleted cells and cells treated with low-dose nocodazole. Arrows indicate cortically localized DHC–GFP and arrowheads indicate spindle poles. (d) Histogram showing the relationship between spindle-pole/cortex distance and dynein localization based on data from c for spindle poles moving towards the cell cortex. The numbers in parentheses indicate the average spindle-pole/cortex distance when dynein localizes to the cortex (blue) or is delocalized (red). (e) Fluorescence micrographs (left) and line scan (right) as in a showing GFP–LGN and mCherry–Arp1A localization in cells treated with the Eg5 inhibitor STLC to create monopolar spindles. Scale bars, 10 μm.

We next sought to precisely analyse the effect of the spindle pole on cortical dynein–dynactin localization. We found that this effect is strongly distance dependent. Dynnein localizes to the cortex when the pole-to-cortex distance is greater than ~3 μm, but becomes delocalized when the pole moves to within 2 μm of the cortex (Fig. 1d). In addition, when the position of the spindle pole was manipulated by inducing monopolar spindles using the Eg5 inhibitor STLC, LGN localized generating this pulling force. Low-dose nocodazole treatment, which has been shown to selectively disrupt astral microtubules 4, caused a dose-dependent impairment of spindle oscillations without affecting cortical dynein enrichment (Fig. 1c). Taken together, these results indicate that cortical dynein–dynactin localization, but not LGN, is regulated to correct spindle positioning in HeLa cells by generating asymmetric forces to centre the spindle within the cell.

Figure 1 Dynein and dynactin localize asymmetrically to the cell cortex during metaphase. (a) Left, time-lapse images from a clonal HeLa cell line stably expressing GFP–LGN and mCherry–Arp1A. LGN localizes to the cell cortex before Arp1A, and exhibits symmetric localization. Arp1A exhibits asymmetric localization to the cell cortex when the spindle is mis-positioned (arrowhead). Right, graph of relative fluorescence intensity for a line scan of the indicated lines showing the spatial distribution of LGN and Arp1A. (b) Schematic showing the dependency relationships and symmetric–asymmetric behaviour for cortical localization. (c) Kymographs showing DHC–GFP and chromosomes (Hoechst) generated from time-lapse movies at 5 min intervals as indicated illustrating the oscillation of the spindle and its effect on cortical dynein localization in MG132-arrested control cells, LGN-depleted cells and cells treated with low-dose nocodazole. Arrows indicate cortically localized DHC–GFP and arrowheads indicate spindle poles. (d) Histogram showing the relationship between spindle-pole/cortex distance and dynein localization based on data from c for spindle poles moving towards the cell cortex. The numbers in parentheses indicate the average spindle-pole/cortex distance when dynein localizes to the cortex (blue) or is delocalized (red). (e) Fluorescence micrographs (left) and line scan (right) as in a showing GFP–LGN and mCherry–Arp1A localization in cells treated with the Eg5 inhibitor STLC to create monopolar spindles. Scale bars, 10 μm.
**Figure 2** Plk1 negatively regulates the localization of cortical dynein. (a) Fluorescence micrographs showing DHC–GFP and DNA (Hoechst) localization in cells treated with the Eg5 inhibitor STLC to create monopolar spindles with or without inhibition of Plk1 (BI2536). (b) Fluorescence micrographs showing the localization of DHC–GFP or GFP–LGN (bottom), DNA (Hoechst, top) and the indicated membrane-targeted mCherry-tagged constructs (top). Membrane-targeted Plk1, but not Plk1 mutants, disrupt cortical dynein localization, but not LGN. (c) Histogram showing quantification of the data in (a) for the frequency of cortical dynein localization ± s.d. for the indicated conditions. Control, n = 34; wild type, n = 50; kinase dead, n = 32; polo-box mutant, n = 19; Aurora A, n = 31; TPX2, n = 11. ** indicate that the Plk1-targeted cells are statistically different from the other conditions with a 99.9% confidence interval on the basis of a z test for a difference between proportions. (d) Kymographs showing cortical dynein localization and spindle oscillations as in Fig. 1c for the indicated membrane-targeted fusions. (e) Western blots showing the presence of selected proteins in the samples in Table 1. (f) Model indicating the effect of Plk1 and spindle-pole proximity on the localization of cortically localized dynein downstream of LGN. MTs, microtubules. Scale bars, 10 µm.

to both sides of the cell cortex, whereas Arp1A accumulates only at the cell cortex distal from the spindle pole (Fig. 1e). Together, these data indicate that the spindle pole negatively regulates the cortical localization of dynein–dynactin downstream of LGN.

The proximity of the spindle pole to the cell cortex creates a precise change in dynein localization, indicating the presence of a signal emanating from the spindle pole. We reasoned that a spindle-pole-localized kinase may generate signals to regulate cortical dynein–dynactin localization. Indeed, we found that inhibiting Plk1 activity (using BI2536 treatment) in STLC-treated cells allowed dynein to localize to the cell cortex in proximity of the spindle pole (Fig. 2a). Plk1 inhibition has pleiotropic effects on spindle structure, preventing us from analysing the effect of Plk1 inhibition on spindle oscillations. Therefore, as a complementary approach, we artificially targeted Plk1 to the plasma membrane. Membrane-targeted Plk1 severely reduced the level of cortical dynein accumulation and eliminated spindle oscillations (Fig. 2b–d). In contrast, targeting of the spindle-pole-localized Aurora A kinase or the Aurora-A-associated domain of TPX2...
(1-43 amino acids; ref. 11) to the plasma membrane had no effect on cortical dynein localization (Fig. 2c and Supplementary Fig. 8c). The effect of membrane-targeted Plk1 was dependent on its kinase activity as cortical dynein localization was unchanged following membrane targeting of kinase-dead or polo-box mutants of Plk1 (Fig. 2b,c and Supplementary Fig. 8c). LGN remained localized to the cell cortex in the cells expressing membrane-targeted Plk1 (Fig. 2b), consistent with the observed effect of spindle-pole proximity in controlling dynein–LGN localization, but not LGN (Fig. 1a).

To define the biochemical interactions that underlie the regulation of asymmetric dynein localization during mitosis, we isolated GFP–LGN from HeLa cells arrested in mitosis with nocodazole. Using mass spectrometry, we defined the complete set of interacting proteins identified in these purifications, but not unrelated controls. GFP–LGN co-purified with NuMA, as well as the dynactin complex and dynein (Table 1). Interestingly, if the isolated GFP–LGN complexes were incubated in the presence of Plk1 and ATP, this resulted in the disassociation of dynactin–dynein from the LGN–NuMA complex.

| Protein                      | GFP–LGN immunoprecipitate (percentage of sequence coverage) | M(K) |
|------------------------------|--------------------------------------------------------|------|
| LGN                          | 63.9                                                   | 63.0 | 76.6 |
| NuMA                         | 56.7                                                   | 56.9 | 238.3|
| Dynactin1, p150              | 7.4                                                    | 141.6|
| Dynactin2, p50               | 42.1                                                   | 44.8 |
| Dynactin3                    | 19.3                                                   | 19.4 |
| Dynactin, Arp3A              | 25.3                                                   | 42.6 |
| Dynein 1 heavy chain 1       | 4.8                                                    | 532.4|
| Dynein 1 light intermediate chain 1 | 16.6                                               | 56.6 |
| Dynein 1 light intermediate chain 2 | 3.7                                                 | 54.0 |

Summary of mass spectrometry data showing the percentage of sequence coverage for the proteins that co-purify with GFP–LGN, but not controls, in the presence or absence of treatment with Plk1 and ATP.

doses of nocodazole to depolymerize the spindle (Fig. 3a). In each case, LGN was excluded from the cortex in regions close to chromosomes. For example, LGN showed a uniform distribution throughout the cell cortex in nocodazole-treated cells in which the chromosomes collapse to the centre of the cell (Fig. 3a). However, in cases where the chromosome mass is located near the cell cortex, we found that LGN localization was locally disrupted (Fig. 3a). LGN is also selectively excluded from the cell cortex in regions proximal to even a single misaligned chromosome (Fig. 3b). A similar sensitivity of LGN to chromosome position was observed in Rpe1 cells (Supplementary Fig. 54b,c). To define the properties of this chromosome-derived signal, we measured the distance between chromosomes and the cell cortex. We found that LGN is excluded from the cell cortex in cases where a chromosome is within ~2 µm (Fig. 3c).

To identify the molecules responsible for this chromosome-derived signal, we investigated two possible chromosome-localized signalling molecules: Aurora B kinase12 and RanGTP (refs 13,14). Inhibition of Aurora B kinase activity by the specific inhibitor ZM447439 had no effect on cortical LGN localization (Supplementary Fig. 5d4). In contrast, transiently transfected dominant-negative mCherry–RanT24N (ref. 15) allowed LGN to localize homogeneously throughout the cell cortex including to regions near chromosomes (Fig. 3d–f). Under these conditions, transfected cells entered mitosis and aligned their chromosomes, indicating that the disruption of interphase nuclear transport by RanT24N expression did not block cell-cycle progression during the time course of these experiments. To investigate the effect of the Ran gradient in the absence of potential prior defects in nuclear–cytoplasmic transport, we used the tsBN2 cell line, which contains a temperature-sensitive mutant of the RanGEF RCC1 (ref. 16). In tsBN2 cells arrested in mitosis at the permissive temperature using nocodazole, LGN is restricted from the cortex when chromosomes are close to the edge of the cell (Fig. 3g). In contrast, when arrested tsBN2 cells were subsequently shifted to the restrictive temperature, LGN localized throughout the cell cortex even when chromosomes are located close to the cortex (Fig. 3g and Supplementary Fig. 54e).

The temperature shift does not affect cortical LGN localization in the parental BHK cell line (Supplementary Fig. 54f). Taken together, these results indicate that a chromosome-derived RanGTP gradient negatively regulates cortical LGN localization to generate a bi-lobed distribution. Previous work demonstrated that the RanGTP gradient contributes to spindle assembly, particularly in the absence of centrosomes14. However, specific roles for this gradient in somatic cells have remained elusive. The implication of the RanGTP gradient in negatively regulating cortical LGN represents an intriguing new activity for this chromosome-derived signal.

To investigate the functional contributions of restricted LGN localization to spindle orientation, we used ‘L’-shaped fibronectin-patterned coverslips (Fig. 3h). Recent work has established that HeLa cells orient their spindles along the hypotenuse of the ‘L’ with high efficiency4. Although >70% of control cells orient their spindle (Fig. 3i,j), depletion of LGN or the dynactin subunit p150 randomized this orientation (Fig. 3i). Consistent with the regulation of cortical dynein localization by Plk1 described above, membrane-targeted Plk1 disrupted proper spindle orientation (Fig. 3i). Importantly, expression of mCherry–RanT24N also severely perturbed proper spindle orientation (Fig. 3i). These data indicate that the bipolar pattern of...
LGN localization downstream of the RanGTP gradient is required to maintain the spindle orientation axis.

We next sought to define the mechanisms by which LGN is regulated by the chromosome-derived RanGTP gradient. Previous work demonstrated that NuMA interacts with the amino terminus of LGN (LGN-N) and is required to recruit LGN to the plasma membrane by facilitating the interaction of the LGN carboxy terminus (LGN-C) with Gti (Fig. 4a). Importantly, NuMA is an established downstream target of the Ran gradient. Consistent with this, we identified importin-α and -β in affinity purifications of LGN (LGN-N) and is required to recruit LGN to the plasma membrane by facilitating the interaction of the LGN carboxy terminus (LGN-C) with Gti (Fig. 4a). Importantly, NuMA is an established downstream target of the Ran gradient.

![Figure 3](image_url)
of the nuclear localization sequence (NLS)-containing GFP-tagged NuMA C terminus (NuMA-C) from HeLa cells (data not shown), although we note that the importin proteins are occasionally found at low coverage in control purifications. NuMA is released from importin-β by RanGTP, which has been suggested to promote spindle assembly in the vicinity of chromosomes. In contrast to the effect of Plk1 on the GFP–LGN complex in the presence of GTP-loaded Ran<sup>Q69L</sup>, a GTP-hydrolysis-defective mutant of Ran, did not disrupt this complex (Supplementary Table S1). Thus, RanGTP probably acts by locally altering the ability of the NuMA–LGN complex to interact with the membrane instead of regulating the NuMA–LGN interaction downstream of LGN, and the chromosome-derived RanGTP gradient negatively regulates NuMA–LGN distribution. Together, these two intrinsic signals act to control spindle position and orientation in symmetrically dividing cells.

To analyse the interactions and regulation of LGN, we assessed the localization of the N-terminal and C-terminal regions of LGN (Fig. 4b and Supplementary Fig. S5a,b). In contrast to full-length LGN, LGN-N localizes to spindle poles during mitosis and to the nucleus in interphase, similar to NuMA (ref. 9; Supplementary Fig. S5b). LGN-C shows increased interactions with G<sub>αi</sub> and lacks NuMA binding (Supplementary Table S1), and localized throughout the cell cortex and to retraction fibres (Fig. 4b), identically to G<sub>αi</sub>1 (Supplementary Fig. S5c) including near misaligned chromosomes (Fig. 4c). Interestingly, when NuMA-C was fused directly to LGN-C, this in-frame fusion exhibited similar restricted cortical localization to full-length LGN in metaphase even in the absence of endogenous LGN (Fig. 4d). However, on elimination of the importin-β-binding NLS in NuMA, this fusion showed more homogeneous mitotic localization (Fig. 4d) and also localized to retraction fibres (Supplementary Fig. S5d). These data indicate that chromosome-derived RanGTP acts at least in part to locally regulate binding of importin-β to the NuMA NLS, altering the ability of the LGN–NuMA complex to target to the membrane. However, it is possible that additional downstream targets for RanGTP to control LGN distribution may exist. We also note that others have suggested recently that Ran is required for spindle orientation in artificially induced asymmetric cell divisions in <em>Drosophila</em> cells. However, in this case, the authors suggested that Ran plays a global positive role in promoting Mud–Pins (the NuMA–LGN counterparts) recruitment to membranes instead of locally inhibiting localization downstream of a chromosome-derived RanGTP gradient.

Defining the mechanisms that control spindle position and orientation is key to understanding cell division. Here, we demonstrated that cortical dynemin localization is regulated by two distinct intrinsic signals to correct spindle position and orientation in symmetrically
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METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturecellbiology

Note: Supplementary Information is available on the Nature Cell Biology website

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AUTHOR CONTRIBUTIONS

T.K. and I.M.C. designed the experiments. T.K. carried out all of the experiments. I.M.C. assisted with some experiments including protein purification and mass spectrometry. T.K. and I.M.C. analysed the data and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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Methods

Cell culture and siRNA transfection HeLa cells, Rpe1 cells and tsBN2 cells were maintained as described previously11,12. Clonal cell lines stably expressing GFP14 were generated as described previously13,14. HeLa cells expressing mouse DHC–GFP were obtained from MitoCheck28. GFP–LGN and DHC–GFP cell lines functionally complement depletion of the corresponding endogenous protein based on dynein recruitment and spindle orientation, respectively (Supplementary Fig. S5c,e; data not shown). To inactivate RCC1, tsBN2 cells were cultured at 39.7°C for 1.5–3 h. mCherry–Ran1750 and membrane-targeted mCherry-tagged constructs (‘Mem’ from Neuromodulin; Clontech) were investigated following transient transfection using Effectene (Qiagen). To examine p150-dynactin (sc-56536, BD Transduction Laboratories, 1:500), Gαi isoforms (sc-56535, 1:20; sc-56537, 1:20), and membrane-targeted mCherry-tagged GFP were obtained from MitoCheck. For RNAi experiments on patterned coverslips, cells were synchronized using a double thymidine block before plating.

To inactivate NuMA, cells were plated on L-patterned fibronectin-coated coverslips (Santa Cruz Biotechnology, 1:20) and NuMA (ab36999, Abcam, 1:1,000). To examine p150-dynactin, cells were incubated for 1–3 h with drugs at the following concentrations: STLC, 100 nM; nocodazole, 100 nM (high dose) or 10–20 nM (low dose); ZM447439, 2 μM; BI2536, 10 μM; MG132, 20 μM; thymidine, 2 mM.

RNA-mediated interference (RNAi) experiments were conducted using RNAi MAX transfection reagent (Invitrogen) according to the manufacturer’s guidelines. Pools of 4 pre-designed short interfering RNAs (siRNAs) against LGN–GPM52 (5′-GAAUUGCCAGCAAGACAUUA-3′, 5′-CUCUGAGAGGAAUUGCUAA-3′, 5′-CACAGUGUAAACUUCUGCUA-3′, 5′-UGAAGGGGUUUGCUACUAU-3′), p50-DCTN1 (5′-CUGGAGGGUGUAUCGUAAA-3′, 5′-GAAGAUCGAGAGACAGUUA-3′, 5′-GCCUAUGCCUCCGUICUCAU-3′, 5′-CGAGCCUCACUACGCUACUAU-3′) and DHC–DYNCH1 (5′-GAUCAAGAUGGCAAUUAU-3′, 5′-GAGAAGACUCUGGGAUAUUA-3′, 5′-GACACGUGGUAAGUGUAA-3′, 5′-CGGAAUGUCAUGAAGCAUGUU-3′, 5′-CCGAAUGGUCAUGAAGCAGUU-3′, 5′-CUUGGAGGCAUCAUGUACUGUU-3′; ref. 9) and the three Gco isoforms (5′-CCGAAGUGCAAAGAAGCAUGU-3′, 5′-CUUAGGCGGCAUCAUGCUACUAU-3′; ref. 8) and a non-targeting control were transfected into tsBN2 cells.

RNAi rescue experiments with LGN, a single siRNA (5′-GAAUCUACACGCAGCAUCUA-3′) was used and the target sequence on the plasmid was mutated to be insensitive to this siRNA. Clonal cell lines stably expressing mouse DHC–GFP were obtained from Dharmacon. For RNAi rescue experiments with LGN, a single siRNA was designed to target the LGN sequence. A single siRNA targeting NuMA (5′-GAUCAAGAUGGCAAUUAU-3′, 5′-GAGAAGACUCUGGGAUAUUA-3′, 5′-GACACGUGGUAAGUGUAA-3′, 5′-CGGAAUGUCAUGAAGCAUGUU-3′, 5′-CCGAAUGGUCAUGAAGCAGUU-3′, 5′-CUUAGGCGGCAUCAUGCUACUAU-3′; ref. 8) and a non-targeting control were transfected into tsBN2 cells.

Images were acquired on a DeltaVision Core microscope (Applied Precision) equipped with a CoolSnap HQ2 CCD (charge-coupled device) camera. Thirty to forty z sections were acquired at 0.5-μm steps using an Olympus 40×, 1.35 NA U-PlanApo, 20×, 0.75 NA U-Apo or 4×, 0.16 NA U-PlanApo objective with 1× 1 binning. Images were deconvolved using the DeltaVision software. Equivalent exposure conditions and scaling was used as appropriate. Fluorescence intensity and distance measurements were analysed using Softworx (Applied Precision) and Metamorph (Molecular Devices). Kymographs were generated using Photoshop (Adobe).

Affinity purifications and mass spectrometry. GFP10,11-tagged LGN was isolated from HeLa cells as described previously25, with 1% Triton X-100 added to prepare the cell lysate. Purified proteins were identified by mass spectrometry using an LTQ XL ion trap mass spectrometer (Thermo) using MudPIT and SEQUEST software as described previously27. Plk1 phosphorylation was conducted on GFP–LGN-isolated beads for 60 min at 30°C using 1 μg Plk1 (Invitrogen). GST–Ran297 was purified from bacteria, loaded with GTP (ref. 28) and incubated at 10 μM with the LGN purification in the cell lysate and first wash.

Statistics To determine the significance between the data obtained for two experimental conditions, a Student t-test (GraphPad Software) or z test (McCallum Layton) was used as indicated in the figure legends.

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Figure S1 Analysis of dependency relationships between cortically localized proteins. (a) Table summarizing the tested dependency relationships for cortical localization (b) Analysis of the interdependent localization for dynein (DHC-GFP), dynactin (GFP-Arp1A), and GFP-LGN to the cell cortex during metaphase following RNAi-based depletion of the selected proteins. (c) Images of DHC-GFP in control cells or following LGN depletion. LGN is required for the cortical localization of dynein, but does not affect kinetochore or spindle localized dynein. (d) Summary of dependency relationships. (e) Images of GFP-LGN expressing cells in either controls, cells depleted for the three Gαi isoforms, or cells depleted for NuMA. LGN localization requires Gαi and NuMA. (f) Images of DHC-GFP, Gαi, and NuMA in control cells and the indicated depletions. Scale bars, 10 μm.
Figure S2 Analysis of asymmetric cortical localization. (a) Top, immunofluorescence images showing the co-localization of NuMA and DHC-GFP. Bottom, line scans for the indicated lines showing the symmetric localization of NuMA and the asymmetric localization of dynein to the cell cortex. (b) Immunofluorescence images and line scans as in (a) showing the symmetric cortical localization of GFP-LGN and the asymmetric localization of the dynactin subunit p150. (c) Top, Gαi displays symmetric localization to the cell cortex similar to LGN, but localizes more homogenously throughout the membrane. Bottom, Gαi, but not LGN, localizes to retraction fibers. Immunofluorescence images of cells expressing GFP-LGN probed with anti-Gαi antibodies. (d) Cortically-localized dynein is dynamically redistributed during spindle movement. Top, images from a time lapse sequence DHC-GFP expressing HeLa cells. Bottom, schematic diagrams showing the distribution of DHC-GFP to the cortex in each image. (e) Fluorescent images showing the asymmetric cortical localization of diverse dynein and dynactin subunits. (f) Fluorescent images and a line scan showing the asymmetric localization of the dynein light chain Tctex in Rpe1 cells. Scale bars, 10 μm.
Figure S3 Analysis of cortical dynein localization and Plk1 dependent phosphorylation. (a) A second example of a kymograph showing DHC-GFP localization during spindle oscillations as in Fig. 1d. (b) Left, kymograph from Fig. 1d extended to show dampening of spindle oscillations. Right, trace of spindle pole position in the kymograph together with the average spindle movement during each stage of the oscillation. (c) Top, fluorescent images showing the localization of the indicated mCherry tagged membrane targeted fusions and DNA (Hoechst). Bottom, fluorescent images showing DHC-GFP localization in the corresponding cells. Scale bars, 10 μm. (d) Plk1 directly phosphorylates NuMA and subunits of the dynactin complex. Immunoprecipitated beads for GFP-Arp1A or GFP-LGN were incubated with recombinant His-Plk1 in the presence of [γ-32P]ATP. The incorporation of the radioactive phosphate group was visualized by autoradiography (32P). Specific bands were detected (arrows) corresponding to NuMA and multiple different dynactin subunits. (e) Phosphorylation sites in NuMA (NM_006185), DCTN1-p150 (NM_001135040), and DCTN2-p50 (NM_006400) following Plk1 treatment were identified by mass spectrometry. Phosphorylated Serine or Threonine residues are marked by an asterisk. Only those phosphorylation sites specific to samples with Plk1 added are shown.
**Figure S4** Ran, but not Aurora B, acts as the chromosome-derived signal to restrict LGN localization. (a) Image of GFP-LGN, DHC-GFP, and anti-p150 in cells with tripolar spindles showing the localization to the cell cortex adjacent to the spindle poles and distal to the chromosomes. (b, c) Images of GFP-LGN expressing Rpe1 cells either in controls or following treatment with nocodazole showing the effect of chromosomes on cortical LGN localization. (d) Images of GFP-LGN expressing cells treated with the Aurora B inhibitor ZM447439 for 1.5-2 hrs. In the absence of Aurora B activity, chromosomes still perturb the cortical localization of LGN. (e) Analysis of GFP-LGN localization in Ran temperature sensitive tsBN2 cells. Top, schematic depicting the arrest and temperature shift regimen. Bottom, images of cells from the indicated conditions showing the localization of GFP-LGN to the cell cortex at the restrictive temperature, but not at the permissive temperature. (f) Temperature shift experiments as in (e) for the parental, non-temperature sensitive BHK cell line. Scale bars, 10 μm.
Figure S5 Localization and functional analysis of LGN and LGN truncation mutants. (a) Schematic of LGN showing its binding partners relative to the N- and C-terminal truncations. (b) Localization of the LGN N-terminus (GFP-LGN-N) and NuMA in mitotic and interphase cells. (c) Images showing the co-localization of GFP-LGN-C and Gβ11 to the cell cortex and retraction fibers. (d) Localization of the indicated LGN fragments and fusions in interphase cells (top) or to the cell cortex near the coverslip in mitotic cells (bottom) to assess localization to retraction fibers (arrows). (e) Immunofluorescence images showing the ability of an RNAi-resistant LGN or the LGN C-terminus to complement depletion of endogenous LGN for p150 and NuMA localization. (f) Immunofluorescence images as in (e) showing the ability of full length LGN or LGN-C to rescue dynein localization. Scale bars, 10 μm.
Supplementary Table 1. Mass spectrometry analysis analyzing the effect of Ran-GTP on LGN-NuMA interactions. Summary of mass spectrometry data showing the percent sequence coverage for the proteins that co-purify with GFP-LGN or GFP-LGN C-terminus in the presence or absence of GTP loaded Ran Q69L as indicated.

| Protein                      | GFP IP (% Seq. Coverage) | Molecular Weight (kD) |
|------------------------------|--------------------------|-----------------------|
|                              | GFP-LGN -RanQ69L | GFP-LGN +RanQ69L | GFP-LGN-C |                      |
| LGN                          | 63.9                    | 57.0                  | 33.0      | 76.6                  |
| NuMA                         | 56.7                    | 46.9                  |           | 238.3                 |
| G-alpha i 1                  |                         |                       | 12.7      | 40.5                  |
| G-alpha i 2                  |                         |                       | 17.2      | 40.5                  |
| G-alpha i 3                  | 9.6                     | 18.9                  | 33.6      | 40.5                  |
| Dynactin1, p150              | 7.4                     | 7.8                   |           | 141.6                 |
| Dynactin2, p50               | 42.1                    | 30.3                  |           | 44.8                  |
| Dynactin3                   | 19.3                    | 19.3                  |           | 19.4                  |
| Dynactin, Arp1A              | 25.3                    | 17.3                  |           | 42.6                  |
| Dynein 1 heavy. Chain 1*     | 4.8                     | 6.7                   | 3.4       | 532.4                 |
| Dynein 1 Light Inter. Chain 1| 16.6                    | 13.2                  | 5.9       | 56.6                  |
| Dynein 1 Light Inter. Chain 2| 3.7                     | 6.3                   |           | 54.0                  |

*Indicates presence in some control samples