Developmental Cell, Vol. 5, 829–840, December, 2003, Copyright ©2003 by Cell Press

Drosophila Nonmuscle Myosin II Promotes the Asymmetric Segregation of Cell Fate Determinants by Cortical Exclusion Rather Than Active Transport

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Summary

Cell fate diversity can be achieved through the asymmetric segregation of cell fate determinants. In the *Drosophila* embryo, neuroblasts divide asymmetrically and in a stem cell fashion. The determinants Prospero and Numb localize in a basal crescent and are partitioned from neuroblasts to their daughters (GMCs). Here we show that nonmuscle myosin II regulates asymmetric cell division by an unexpected mechanism, excluding determinants from the apical cortex. Myosin II is activated by Rho kinase and restricted to the apical cortex by the tumor suppressor Lethal (2) giant larvae. During prophase and metaphase, myosin II prevents determinants from localizing apically. At anaphase and telophase, myosin II moves to the cleavage furrow and appears to "push" rather than carry determinants into the GMC. Therefore, the movement of myosin II to the contractile ring not only initiates cytokinesis but also completes the partitioning of cell fate determinants from the neuroblast to its daughter.

Introduction

The asymmetric segregation of cell fate determinants is a fundamental means of generating cell diversity in organisms ranging from bacteria to mammals (reviewed by Knoblich, 2001; Cayouette and Raff, 2002; Ryan and Shapiro, 2003). In the *Drosophila* embryonic CNS, neural precursors (neuroblasts) divide in a stem cell lineage, giving rise to a series of smaller daughter cells, called ganglion mother cells (GMCs). The neuroblast enters another round of asymmetric division, while the GMC divides only once to produce neurons or glia. The choice between a neuroblast and GMC fate is controlled by the asymmetric distribution of cell fate determinants. At least two determinants, the homeodomain protein Prospero (Doe et al., 1991; Vaessin et al., 1991) and the membrane-associated protein Numb (Uemura et al., 1989; Rhyu et al., 1994), are asymmetrically localized to the basal cortex in the neuroblast and segregated to the GMC at cell division. Prospero is localized by an adaptor protein, Miranda (Ikeshima-Kataoka et al., 1997; Shen et al., 1997), while Numb requires the adaptor Partner of Numb (Pon) (Lu et al., 1999).

The localization of cell fate determinants is coordinated with the orientation of the mitotic spindle during asymmetric division. This is directed by an evolutionarily conserved apical protein complex consisting of the PDZ domain proteins Bazooka (Baz) (Kuchinke et al., 1998; Schober et al., 1999; Wodarz et al., 1999) and DmPAR-6 (Petronczki and Knoblich, 2001) and the atypical protein kinase C (DaPKC) (Wodarz et al., 2000). The apical complex recruits Inscuteable (Insc) (Kraut and Campos-Ortega, 1996; Kraut et al., 1996), Partner of Inscuteable (Pins) (Parmentier et al., 2000; Yu et al., 2000), and the heterotrimeric G protein Gαi (Schaefer et al., 2000, 2001). Mutations that disrupt this complex result in misorientation of the mitotic spindle and mislocalization of cell fate determinants (reviewed by Jan and Jan, 2001; Knoblich, 2001).

Two tumor-suppressor proteins, Lethal (2) giant larvae (Lgl) (Gateff, 1978) and Discs large (Dlg) (Woods and Bryant, 1991), are required for the localization of cell fate determinants but not the apical complex (Ohshiro et al., 2000; Peng et al., 2000). Dlg and Lgl are present uniformly around the neuroblast cortex, and Dlg is necessary for cortical Lgl localization. Although Lgl is uniformly distributed at the cortex, it is thought to be inactivated on the apical side of the cell through phosphorylation by aPKC (Betschinger et al., 2003).

The asymmetric localization of determinants changes throughout the neuroblast cell cycle. At interphase, Miranda and Prospero accumulate apically, while Pon and Numb are uniformly cortical. From prophase onward, both complexes form a tight basal crescent. After cytokinesis, Miranda releases Prospero, which then translocates to the GMC nucleus to regulate GMC identity genes (reviewed by Lu et al., 2000). When actin filaments are depolymerized by treatment with latrunculin A, Prospero, Numb, and their adapters no longer localize basally (Broadus and Doe, 1997; Knoblich et al., 1997; Shen et al., 1998; Lu et al., 1999). The dynamic localization of determinants and their dependence on an intact actin cytoskeleton suggest the involvement of a myosin motor. Recently, the *Drosophila* myosin VI Jaguar (Jar) was found to bind to Miranda and be required for its basal localization (Petrisch et al., 2003). Petrisch et al. (2003) infer that Jar transports Miranda to the basal cortex. Jar and Miranda do not clearly colocalize in neuroblasts, leading the authors to suggest that Jar does not anchor Miranda at the basal cortex and that formation of a basal crescent might involve other myosin motors. Moreover, they find that Miranda may form a complex with nonmuscle myosin II (myosin II hereon) and suggest that Jar and myosin II might compete for binding to Miranda. Here we show that myosin II is required for basal crescent formation but by an unexpected mechanism, apical exclusion rather than active transport.

Class II myosins are heterohexamers consisting of a pair of heavy chains (MHCs), which bind to actin and are responsible for myosin's motor activity, a pair of regulatory light chains (RLCs), and a pair of essential light chains (ELCs). Phosphorylation of the RLCs regulates myosin II activity (reviewed by Bresnick, 1999; Matsumura et al., 2001). In *C. elegans*, myosin II is required to localize the PAR proteins and establish cell polarity

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(Guo and Kemphues, 1996; Shelton et al., 1999; Cuenca et al., 2003; Severson and Bowerman, 2003). RNAi of either the *C. elegans* myosin II heavy chain (NMY-2) or its corresponding regulatory light chain (RLC-4) disrupts asymmetric cell division: PAR-3, which normally forms an anterior crescent, expands around the entire cortex, and PAR-2, which is normally found at the posterior cortex, forms a patch rather than a crescent. NMY-2 is present uniformly around the cortex of the zygote and interacts directly with PAR-1, but the mechanism by which it directs the asymmetric localization of PAR proteins is as yet unknown.

The *Drosophila* homolog of NMY-2 is encoded by *zipper* (*zip*) (Kiehart et al., 1989) and its regulatory light chain by *spaghetti squash* (*sqh*) (Karess et al., 1991). Zygotic null mutants for *zipper* (*zip¹*; Young et al., 1993) die as embryos that fail to complete dorsal closure, an acto-myosin-driven movement in which the lateral epidermal sheets meet and "zip up" along the embryonic dorsal midline.

Zipper binds directly to Lgl (Strand et al., 1994), which is essential for basal protein targeting (Ohshiro et al., 2000; Peng et al., 2000). Furthermore, genetic interactions between Zipper and Lgl have led to the suggestion that Zipper has a negative effect on basal crescent formation (Ohshiro et al., 2000; Peng et al., 2000). However, no defects in asymmetric localization are observed in *zipper* zygotic mutants (*zip¹*). As reported previously by Ohshiro et al. (2000) and Peng et al. (2000), we find no change in Miranda localization in *zip¹* neuroblasts. Yet, myosin II is supplied maternally, which can mask the loss of zygotic myosin II. In fact, the level of myosin II protein is not detectably reduced in *zip¹* mutant neuroblasts until stage 17 (Phelps, 2000; data not shown). Until now it has been unclear what, if any, role myosin II has in asymmetric division.

Here we show that myosin II regulates the basal targeting of cell fate determinants in dividing neuroblasts, not by direct transport but by cortical exclusion. We demonstrate that myosin II is activated by Rho kinase and restricted to the apical neuroblast cortex by Lgl. At prophase and metaphase, myosin excludes determinants apically and confines them to the basal cortex. At anaphase and telophase, myosin shifts toward the cleavage furrow in an Lgl-independent manner and seems to "push" determinants into the GMC. We show that cortical myosin II is essential for the segregation of determinants to the daughter cell but is not required to localize apical proteins nor to orient the mitotic spindle in neuroblasts.

Results

Myosin II Is Required to Localize Determinants to the Neuroblast Cortex

To investigate the role of myosin II in localizing cell fate determinants, we took different approaches to reduce or eliminate myosin activity. As myosin II is required during oogenesis, germline clones of null mutations produce no eggs (Jordan and Karess, 1997). Therefore, we made germline clones of a hypomorphic mutation in *sqh¹*, which reduces the level of light chain transcripts to 5%–10% of wild-type but does not abolish egg laying (Karess et al., 1991). In 44% of *sqh¹* GLC metaphase neuroblasts, cell fate determinants are mislocalized (n = 98; Figure 1). The mutants can be grouped into two classes: severe and mild. In the severe class, localization of the basal complex is disrupted. Miranda is no longer at the cortex but concentrates on the mitotic spindle (25%, n = 98; Figures 1B, 1H, 1K, 1N, and 1O). Prospero also localizes weakly to the spindle microtubules (Figure 1E), and Numb is cytoplasmic (Figure 1H). The mitotic spindles are less focused than their wild-type counterparts (Figure 1O). In the mild class, Miranda and Prospero are partially mislocalized, associating both with the basal cortex and the spindle microtubules (19%, n = 98; Figures 1C, 1F, 1I, and 1L). Numb still localizes to the basal cortex (Figure 1I), although it is more punctate and concentrates over the spindle pole rather than being uniformly distributed in a crescent. Bazooka is at the apical cortex in both the severe and mild classes, although its localization is weaker in the severe mutant class (Figures 1K and 1L).

When the ratio of myosin heavy to light chains is perturbed, for example, when the number of light chains is reduced, myosin heavy chains form inactive aggregates (Edwards and Kiehart, 1996; Jordan and Karess, 1997). We see aggregates in *sqh¹* GLC neuroblasts exhibiting both the mild and severe phenotypes, although the number of aggregates is far greater in the severe class (see below and Supplemental Figure S1 available at http://www.developmentalcell.com/cgi/content/full/5/6/829/DC1). This suggests that there are fewer myosin light chains, and less active myosin, in the severe mutant class. As myosin II is known to organize the actin cytoskeleton (reviewed by Tan et al., 1992), we assayed the integrity of the cortical actin cytoskeleton in neuroblasts of *sqh¹* GLC embryos. In the severe class of *sqh¹* GLC neuroblasts, filamentous actin (F-actin) is discontinuous at the cortex, unlike in wild-type (Figure 1N, compare to Figure 1M). Miranda is found on the mitotic spindle (Figure 1N), presumably because it cannot be anchored at the cortex when the actin cytoskeleton is disrupted.

In 56% of *sqh¹* GLC embryos, cell fate determinants localize normally. This is most likely due to paternal rescue, as *sqh* is contributed zygotically from the paternal X chromosome in 50% of the embryos (see Experimental Procedures). We see no obvious defects in spindle orientation in either the mild or severe class of mutant neuroblasts. Thus, myosin II is required for the localization of basal but not apical proteins and is not required to orient the mitotic spindle.

Rho Kinase Regulates Myosin II Activity in Neuroblasts

Myosin's role in asymmetric division could be solely to maintain the actin cortex, or it might be more specifically involved in localizing cell fate determinants. To distinguish between these two possibilities, we sought to inactivate myosin II during neuroblast cell division without altering the concentration of the protein in the cell or disturbing the actin cytoskeleton. We inhibited Rho kinase, a key regulator of myosin II activity both in vertebrates and invertebrates (Amano et al., 1996; Kimura et

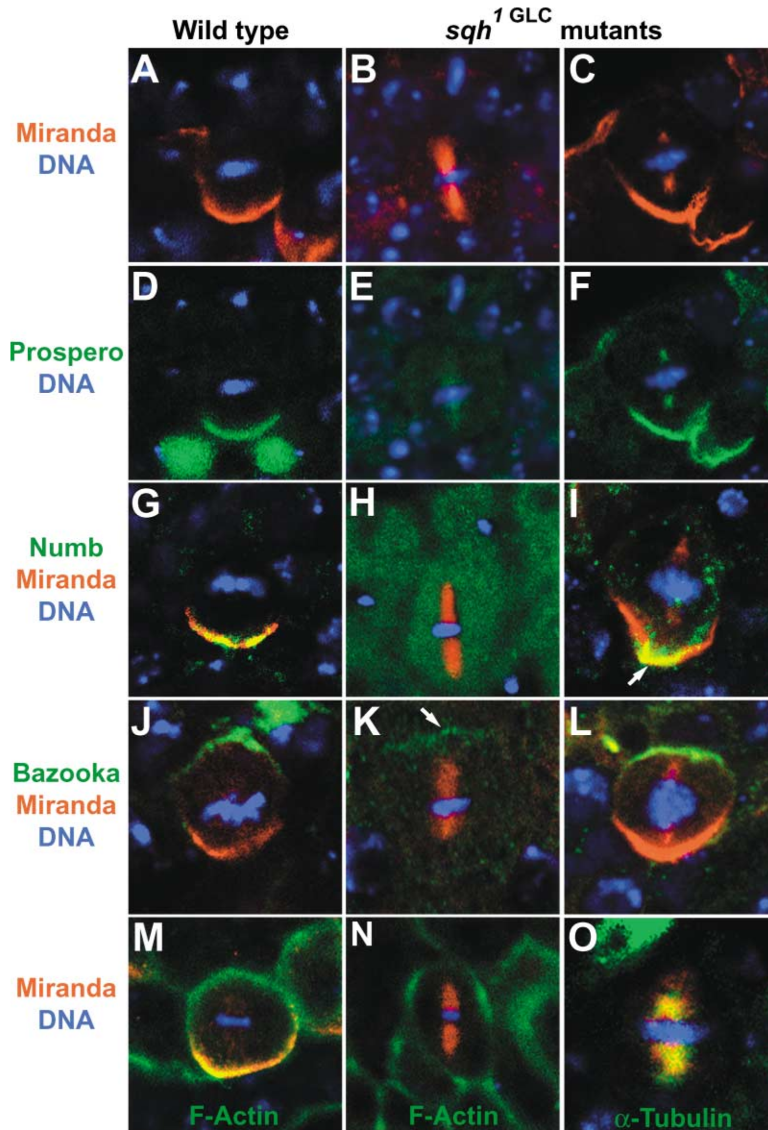

Figure 1. Myosin II Is Required in Neuroblasts to Localize Basal Determinants but Not Apical Proteins

In wild-type metaphase neuroblasts, Miranda (red, [A, G, J, and M]), Prospero (green, [D]), and Numb (green, [G]) form basal cortical crescents. In the severe class of *sqh¹* GLC neuroblasts, Miranda and Prospero redistribute from the cortex to spindle microtubules (Miranda, red, [B, H, K, N, and O]; Prospero, green, [E]), and Numb is cytoplasmic (Numb, green, [H]). In the mild class of *sqh¹* GLC neuroblasts, Miranda and Prospero are partially redistributed from the cortex to spindle microtubules (Miranda, red, [C, I, and L]; Prospero, green, [F]), and Numb still localizes to the basal cortex (green, [I], arrow). In wild-type metaphase neuroblasts, Bazooka (green, [J]) forms an apical cortical crescent. In severe *sqh¹* GLC neuroblasts, Bazooka localizes apically, but its levels are reduced (green, [K], arrow). In mild *sqh¹* GLC neuroblasts, Bazooka localizes normally (green, [L]). In wild-type metaphase neuroblasts, F-actin (green, [M]) is uniformly cortical. In severe *sqh¹* GLC neuroblasts, F-actin (green, [N]) is still present at the cortex but is no longer uniform. In these mutants, Miranda (red, [O]) colocalizes with α-tubulin (green, [O]). Neuroblasts are from stage 10/11 embryos. Apical is up. DNA is labeled in blue.

al., 1996; Winter et al., 2001). In mammals, Rho kinase phosphorylates the RLC on residues Ser19 and Thr18 and in *Drosophila* on Ser21 and Thr20. Phosphorylation at these sites induces a conformational change that enables myosin to assemble into bipolar filaments and increases myosin's actin-dependent ATPase activity (reviewed by Tan et al., 1992). Rho kinase also inhibits myosin phosphatase, thereby preventing dephosphorylation of the RLC (Kimura et al., 1996; Kawano et al., 1999). In *Drosophila* Rho kinase mutants, the level of phosphorylated RLC (Sqh) is greatly reduced, and cortical phospho-myosin is nearly absent (Winter et al., 2001). Consistent with these results, inhibiting Rho kinase activity prevents cortical recruitment of myosin II in syncytial blastoderm embryos (Royou et al., 2002).

To block phosphorylation of Sqh and inactivate myosin II in embryonic neuroblasts, we made use of a specific Rho kinase inhibitor, Y-27632 (reviewed by Narumiya et al., 2000). When embryos are injected at stage 10 with Y-27632, cortical F-actin is continuous (Figures 2J and 2J′), and Bazooka localizes apically (100%, n = 71; Figure 2H), but Miranda, Prospero, and Numb are found around the entire cortex of mitotic neuroblasts (Miranda: 100%, n = 109, Figure 2B; Prospero: 100%, n = 181, Figure 2D; Numb: 100%, n = 69, Figure 2F). Therefore, inactivating myosin II results in mislocalization of basal but not apical factors.

Phosphorylation of Sqh can be mimicked by replacing Thr20 and Ser21 with glutamate (SqhE20E21) (Winter et al., 2001; Royou et al., 2002). Expression of this phosphomimetic form of Sqh, even though constitutively active, can suppress the lethality of Rho kinase mutants (Winter et al., 2001). To test whether SqhE20E21 can restore basal protein localization in neuroblasts, we injected Y-27632 into embryos expressing both wild-type Sqh and SqhE20E21. When Y-27632 is injected into stage 10 embryos, Miranda and Prospero are cleared from the apical side of neuroblasts at telophase and are

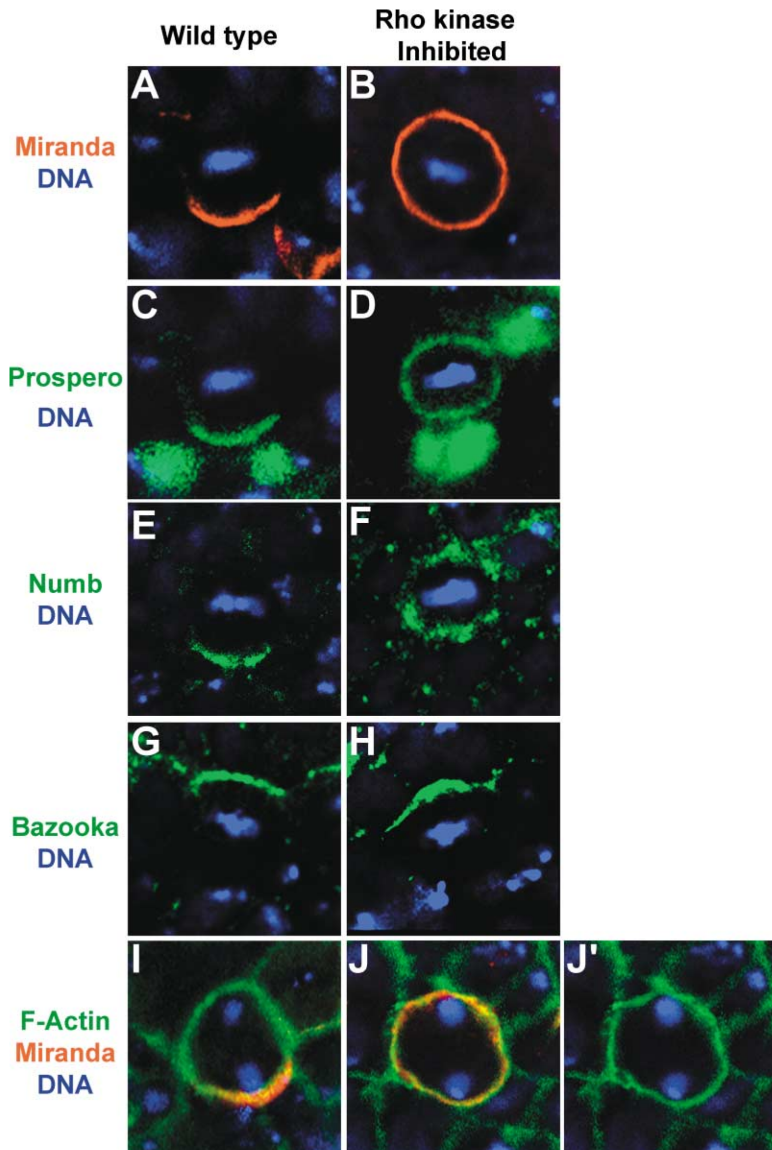

Figure 2. Inhibition of Rho Kinase Prevents Basal Targeting in Neuroblasts

In wild-type metaphase neuroblasts, Miranda (red, [A and I]), Prospero (green, [C]), and Numb (green, [E]) form basal cortical crescents. Inhibition of Rho kinase activity causes Miranda (red, [B and J]), Prospero (green, [D]), and Numb (green, [F]) to localize uniformly around the neuroblast cortex. In wild-type metaphase neuroblasts, Bazooka (green, [G]) localizes to the apical cortex. This localization is not affected by inactivation of Rho kinase (green, [H]). In wild-type metaphase neuroblasts, F-actin (green, [I]) is localized uniformly around the cortex. Rho kinase inhibition has no visible effect on cortical F-actin (green, [J and J′]), whereas Miranda (red, [I and J]) is uniform around the cortex. [J′] is the same image as [J] but shows only the green and blue channels. Neuroblasts are from stage 10/11 embryos. Apical is up. DNA is labeled in blue.

segregated into the GMC (100%, n = 18, Figure 3C). Uninjected Sqh/SqhE20E21-expressing embryos localize Miranda and Prospero normally (data not shown). Therefore, myosin II is a Rho kinase effector in neuroblasts, as it is in the wing (Winter et al., 2001). Inactivating myosin II by inhibition of Rho kinase enables determinants to localize apically as well as basally. Phosphomimetic myosin II can suppress the loss of Rho kinase activity and block the apical localization of determinants at telophase.

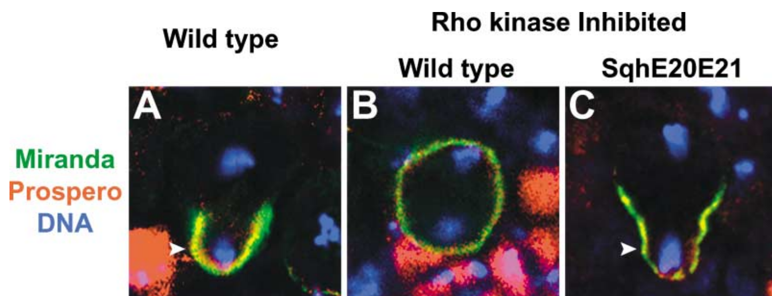

Figure 3. Rho Kinase Regulates Myosin II Activity in Neuroblasts

(A) In wild-type neuroblasts, Miranda (green) and Prospero (red) localize to the cortex of the forming GMC (arrowhead) at telophase. (B) Inhibition of Rho kinase causes Miranda (green) and Prospero (red) to spread around the entire cell cortex. (C) Expression of a phosphomimetic form of myosin II regulatory light chain (SqhE20E21) rescues the loss of Rho kinase activity: Miranda (green) and Prospero (red) are absent from the apical cortex and shifted toward the cortex of the forming GMC in telophase neuroblasts (arrowhead). Neuroblasts are from stage 10/11 embryos. Apical is up. DNA is labeled in blue.

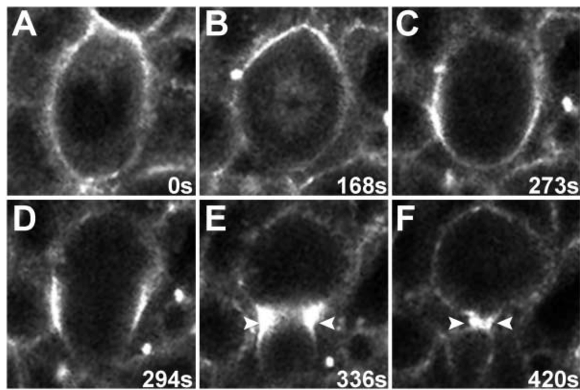

Figure 4. Myosin II Is Asymmetrically Localized in Mitotic Neuroblasts

Neuroblast from live stage 10 embryo expressing a myosin II regulatory light chain (Spaghetti squash) fusion to GFP (Sqh-GFP). (A) At interphase, Sqh-GFP is uniform around the neuroblast cortex and weakly cytoplasmic. (B) At mitosis, Sqh-GFP concentrates at the apical cortex. Sqh-GFP is also weakly detected in the region of the spindle microtubules. (C) The apical accumulation is maintained until anaphase, when Sqh-GFP becomes enriched as a circumferential belt at the equator of the cell. (D, E, and F) At telophase, Sqh-GFP becomes concentrated at the cleavage furrow (arrowheads). Sqh-GFP is expressed from the *sqh* promoter in a *sqh* null mutant background (Royou et al., 2002). In these embryos, the ratio of myosin II regulatory light to heavy chains is maintained. Live embryos were imaged by time-lapse confocal microscopy. Time points are given in seconds (bottom right). Apical is up.

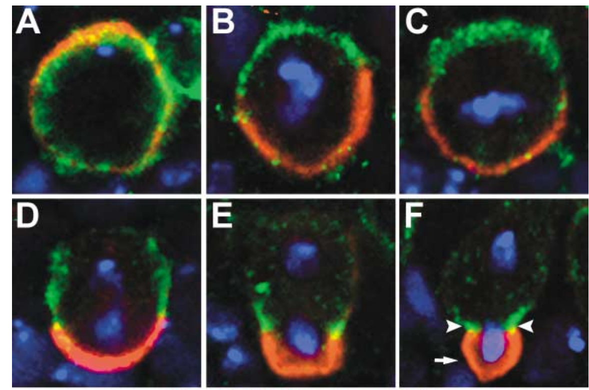

Figure 5. Myosin II and Miranda Localize to Opposite Sides of Mitotic Neuroblasts

(A) At interphase, myosin II (green) is uniform around the cortex and weakly cytoplasmic. Miranda (red) is concentrated at the apical cortex. During prophase (B) and metaphase (C), myosin II concentrates at the apical side of the neuroblast, and Miranda forms a basal crescent. (D) At anaphase, myosin II shifts from the apical cortex and is enriched at the equator. (E and F) At telophase, myosin II concentrates at the cleavage furrow, and Miranda is segregated into the forming GMC. Neuroblasts are from wild-type stage 10 embryos. Myosin II was stained with anti-Zipper. Apical is up. DNA is labeled in blue.

Myosin II Is Asymmetrically Localized in Neuroblasts

We have shown that myosin II is required both to organize the actin cytoskeleton in neuroblasts, enabling determinants to localize to the cortex, and to confine determinants to the basal side of the neuroblast. Myosin II could actively transport Miranda to the basal cortex as has been proposed for myosin VI Jar (Petrisch et al., 2003). If this is the case, we might expect myosin II to move from the apical to the basal cortex during neuroblast division. We analyzed the distribution of myosin II light and heavy chains in living embryos by expressing either Sqh-GFP or GFP-Zipper during neuroblast cell division.

Sqh-GFP is expressed from its own promoter in a *sqh* null mutant background and completely rescues the *sqh* mutant phenotype (Royou et al., 2002). Expression of UAS-GFP-Zipper is driven by GAL4^{V37}. *zip*^{1} mutant embryos, which would normally die as embryos, can be partially rescued by *daughterless*-GAL4 driving UAS-Zipper-GFP. 34% of embryos (n = 128) hatch and undergo larval development.

At interphase, Sqh-GFP localizes at the neuroblast cortex and is found at low levels in the cytoplasm (Figure 4A). At mitosis, Sqh-GFP accumulates in an apical crescent and can also be seen very faintly in the region of the spindle microtubules (Figure 4B). The apical crescent is maintained until anaphase, when Sqh-GFP shifts to the equator and forms a broad belt around the cell (Figure 4C). At late anaphase and telophase, myosin accumulates at the cleavage furrow (Figures 4D–4F; Supplemental Movie S1). GFP-Zipper recapitulates the localization of Sqh-GFP. At interphase, GFP-Zipper is present around the neuroblast cortex and weakly in the cytoplasm. At mitosis, it localizes in an apical crescent until anaphase and telophase, when it shifts to the cleavage furrow (Supplemental Movie S2). Therefore, both the myosin II heavy and light chains first concentrate apically and then move basally during neuroblast cell division, as do Miranda, Prospero, Pon, and Numb (reviewed by Lu et al., 2000).

Myosin II and Miranda Localize to Opposite Sides of Mitotic Neuroblasts

To test further the hypothesis that myosin II transports the Miranda complex to the basal cortex, we investigated whether myosin and Miranda colocalize in neuroblasts. At interphase, endogenous myosin II is cortical and weakly cytoplasmic, while Miranda accumulates at the apical cortex (Figure 5A). Surprisingly, from prophase through metaphase, when myosin II concentrates at the apical cortex, Miranda forms a basal cortical crescent (Figures 5B and 5C). The two proteins abut each other but never overlap extensively. At anaphase, myosin II shifts from the apical cortex to an equatorial belt, and Miranda moves toward the basal pole (Figure 5D). By late anaphase and at telophase, myosin II concentrates at the cleavage furrow, and Miranda is segregated into the GMC (Figures 5E and 5F). We obtain similar results when comparing GFP-Zipper with Miranda (Supplemental Figure S2). Therefore, myosin II and Miranda occupy virtually exclusive domains, arguing against a direct transport mechanism. These results together with our data that inactivating myosin II enables Miranda to localize around the entire neuroblast cortex suggest that myosin excludes Miranda from the apical cortex of the neuroblast rather than carrying it to the basal cortex.

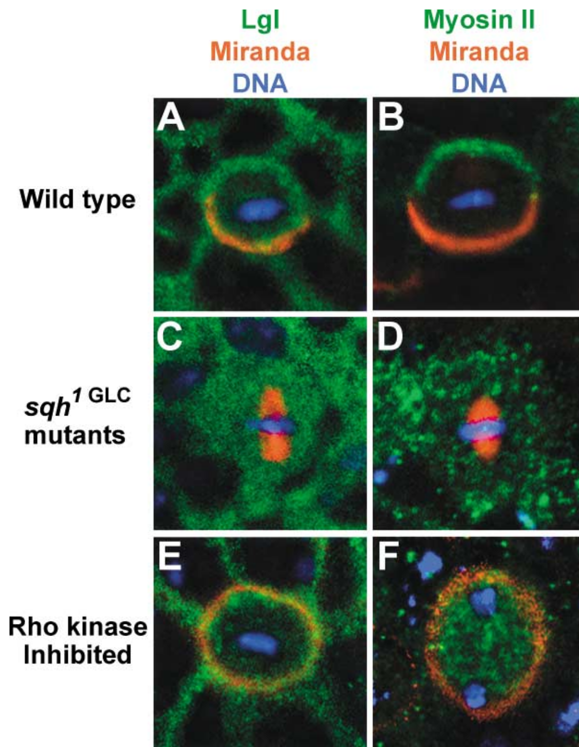

Figure 6. Localization of Miranda but Not Lgl Requires Active Myosin II

(A and B) In wild-type metaphase neuroblasts, myosin II (green, [B]) concentrates at the apical cortex, and Miranda (red) forms a basal crescent, whereas Lgl (green, [A]) localizes around the entire cortex. (C and D) In severe *sqh*$^{1\ GLC}$ neuroblasts, cortical F-actin is no longer uniform (see Figure 1N), myosin II (green, [D]) forms aggregates throughout the cytoplasm, Miranda (red) localizes on spindle microtubules, and Lgl (green, [C]) is cytoplasmic. (E and F) When myosin II activity is blocked by inhibition of Rho kinase, cortical F-actin remains intact (see Figure 2J′), Lgl localization is normal (green, [E]), but myosin II (green, [F]) spreads throughout the cytoplasm, and Miranda (red) becomes uniformly cortical. Neuroblasts are from stage 10 embryos. Myosin II was stained with anti-Zipper. Apical is up. DNA is labeled in blue.

Localization of Miranda but Not Lgl Requires Active Myosin II

Myosin II binds directly to the tumor suppressor Lgl (Strand et al., 1994), which has been shown to regulate the localization of basal but not apical factors in neuroblasts. In *lgl*$^{1\ GLC}$ mutant neuroblasts, Miranda concentrates on spindle microtubules and is found weakly around the entire cell cortex (Ohshiro et al., 2000; Peng et al., 2000; Figure 8A). As this phenotype is similar to *sqh*$^{1\ GLC}$ mutant neuroblasts, we tested whether the loss or inactivation of myosin II disrupts Lgl localization.

In wild-type neuroblasts, Lgl localizes uniformly around the cortex (Ohshiro et al., 2000; Peng et al., 2000; Betschinger et al., 2003; Figure 6A). In *sqh*$^{1\ GLC}$ neuroblasts, Lgl is cytoplasmic rather than cortical (Figure 6C). In these neuroblasts, myosin heavy chains form inactive cytoplasmic aggregates (Figure 6D), and F-actin is no longer uniformly cortical (Figure 2N). The loss of cortical myosin II and the resultant disruption of the actin cytoskeleton precludes Lgl and Miranda from localizing to the cortex (Figures 6C and 6D).

We have shown that myosin II can be inhibited, but the actin cortex maintained, after treatment with a specific Rho kinase inhibitor. In these neuroblasts, myosin II is no longer accumulated at the apical cortex and falls into the cytoplasm (Figure 6F), Miranda spreads around the entire cortex (Figures 6E and 6F), but Lgl is still cortical (Figure 6E). We conclude that myosin II activity is not required to maintain Lgl at the cortex when the actin cytoskeleton is intact. However, cortical Lgl is insufficient to localize Miranda basally. Basal Miranda localization requires active myosin II.

Myosin II Apical Localization Depends on Inactivation of Lethal (2) Giant Larvae

Genetic interactions and immunostainings suggest that Lgl can inhibit the assembly of myosin filaments and maintain myosin II in a noncontractile form (Strand, 1998; De Lorenzo et al., 1999). Phosphorylation of Lgl blocks its interaction with myosin II in vitro (Kalmes et al., 1996), suggesting a means by which Lgl could selectively regulate myosin activity. Recently, it has been shown that Lgl phosphorylation is mediated by aPKC (Betschinger et al., 2003; Plant et al., 2003), which is anchored at the apical cortex of neuroblasts by the Bazooka/DmPar6 complex. Therefore, aPKC can phosphorylate and inactivate Lgl apically but not basally. If active Lgl inhibits myosin II, then myosin should be active apically where Lgl is phosphorylated. Conversely, myosin should be inactive basally where Lgl is active (Figure 8A).

To test this hypothesis, we assayed myosin II localization in embryos lacking both maternal and zygotic Lgl. In metaphase *lgl*$^{1\ GLC}$ neuroblasts, myosin no longer concentrates apically but is found uniformly around the cortex (100%, $n = 25$; Figures 7A and 7A′). This supports our model that myosin is excluded basally due to the activity of Lgl. In the absence of Lgl, myosin localizes uniformly around the entire cortex. Miranda is almost entirely excluded from both the apical and basal cortex (Figures 7A and 8B, left), although myosin appears to be somewhat less efficient at blocking Miranda when it is not concentrated apically.

We also assayed myosin II localization in embryos expressing a nonphosphorylatable form of Lgl. In *Drosophila*, Lgl is phosphorylated on Serine residues 656, 660, and 664 by aPKC (Betschinger et al., 2003). Converting these three residues to Alanines generates a constitutively active molecule that can no longer be repressed apically by aPKC (Lgl-3A; Betschinger et al., 2003). In embryos expressing Lgl-3A, myosin II is excluded from the neuroblast cortex and falls into the cytoplasm (100%, $n = 15$ metaphase neuroblasts; Figures 7C and 7C′). Constitutively active Lgl appears to block myosin activation and prevent the formation of myosin filaments. As a result, myosin can no longer bind to cortical actin, and Miranda is free to bind to the entire neuroblast cortex (Figures 7C and 8B, right).

In *lgl*$^{1\ GLC}$ embryos (Figures 7B and 7B′) and Lgl 3A-expressing embryos (Figures 7D and 7D′), myosin II still accumulates at the cleavage furrow of telophase neuroblasts. Myosin seems to be recruited from the cytoplasm

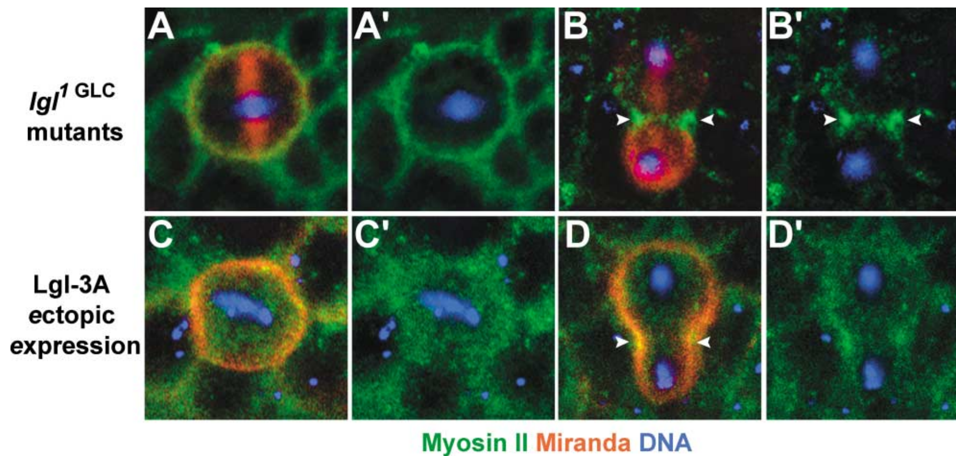

Figure 7. Lgl Regulates Myosin II Apical Localization

(A and A′) In *lgl*^{1} ^{GLC} metaphase neuroblasts, myosin II (green) localizes uniformly around the cortex. Miranda (red, [A]) concentrates along the spindle microtubules and is weakly uniform around the cortex. (B and B′) At telophase, myosin II (green) concentrates at the cleavage furrow (arrowheads), and Miranda (red, [B]) is segregated into the GMC, although it still accumulates at microtubules. (C and C′) In embryos expressing a nonphosphorylatable, constitutively active Lgl (Lgl3A), myosin II (green) spreads throughout the cytoplasm of metaphase neuroblasts, and Miranda (red, [C]) is uniformly cortical. (D and D′) At telophase, myosin II (green) concentrates at the cleavage furrow (arrowheads), and Miranda (red, [D]) is present around the cortex of both the neuroblast and the forming GMC. Neuroblasts are from stage 10 embryos. Myosin II was stained with anti-Zipper. Apical is up. DNA is labeled in blue. (A′), (B′), (C′), and (D′) show green and blue channels only.

to the cleavage furrow in the Lgl-3A-expressing neuroblasts. This suggests that myosin II might be regulated in three separable steps. First, myosin accumulates at the apical cortex at metaphase, a localization that depends on inactivation of Lgl. Second, cortical myosin moves to the equator at anaphase. Third, cortical and cytoplasmic myosin accumulates at the cleavage furrow at telophase. The movement of myosin and accumulation at the furrow is Lgl independent. In *lgl*^{1} ^{GLC} neuroblasts, where myosin II is cortical (Figure 7A′, compare with 7C′), Miranda is efficiently segregated to the GMC (Figure 7B, compare with 7D). However, in neuroblasts expressing Lgl-3A, where myosin is cytoplasmic (Figure 7C′), Miranda is present around the entire cortex of both the neuroblast and the GMC (Figure 7D). Therefore, cortical myosin is required for telophase rescue (Peng et al., 2000; Cai et al., 2001).

Discussion

Myosins have been shown to play a role in asymmetric cell division in yeast, nematodes, and flies. Here we show that the *Drosophila* class II nonmuscle myosin regulates the asymmetric segregation of cell fate determinants during neural stem cell divisions. We propose that myosin II modifies the actin cytoskeleton to exclude determinants from the apical cortex and that myosin localization is regulated by Rho kinase and Lgl.

Myosin II Organizes the Actin Cytoskeleton

Class II myosins are barbed end-directed motors that form bipolar filaments. The filaments bind actin and initiate contraction when the two ends of the bipolar filament pull in opposite directions (reviewed by Tan et al., 1992). Myosin's mode of action makes it unlikely that myosin II could transport cargo from one side of the cell to the other, except perhaps by progressive contraction along the cortex. The lack of colocalization of myosin II and Miranda in neuroblasts further implies that myosin II does not transport Miranda directly. Our data suggest, first, that myosin II is required to maintain an intact cortical actin cytoskeleton and, second, that active myosin modifies the actin cytoskeleton at the apical cortex to exclude Miranda binding. The *C. elegans* myosin II may act in a similar fashion, as it appears to limit PAR-3 to the anterior of the zygote (Cuenca et al., 2003; Severson and Bowerman, 2003).

Myosin II Is Required to Localize Determinants to the Basal Cortex

Treatment of zygotic *lgl* mutants with 25 mM or 50 mM BDM, a supposed general myosin inhibitor, inferred that two different myosins were involved in localizing determinants: myosin II to inhibit basal targeting and a second unknown myosin to promote basal targeting (Ohshiro et al., 2000; Peng et al., 2000). These conclusions must be treated with caution, as recent results have shown that nonmuscle myosins are not inhibited by BDM (Cheung et al., 2002). Instead, BDM appears to block actin polymerization at the leading edge of mammalian cells, in particular by delocalization of proteins such as the Arp2/3 complex, WAVE, and VASP (Yarrow et al., 2003). A specific inhibitor of mammalian myosin II, blebbistatin, has no effect on *Drosophila* cells (Straight et al., 2003).

We have taken several alternative approaches to inactivate myosin II in neuroblasts. First, we analyzed germline clones of Sqh. In severe *sqh*^{1} ^{GLC} embryos, levels of the regulatory light chain are greatly reduced from early development, and the heavy chain is found only in inactive aggregates. The actin cytoskeleton is disrupted, and neither Lgl nor Miranda localize to the cortex. Miranda

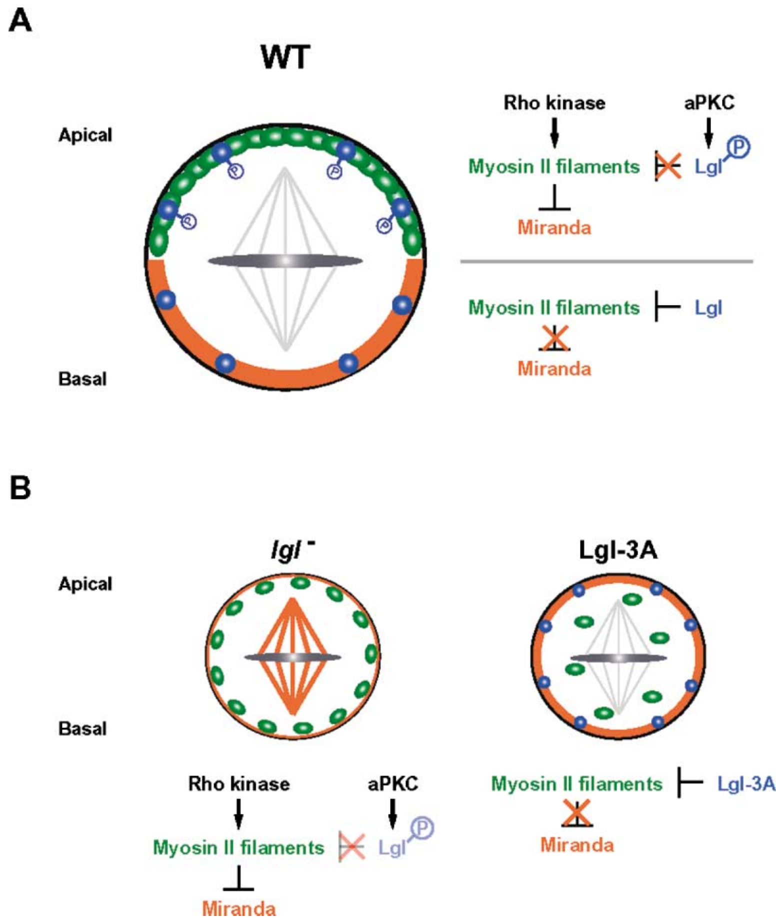

Figure 8. Myosin II Regulates Asymmetric Segregation of Cell Fate Determinants in Dividing Neuroblasts

(A) Asymmetrically localized myosin II may modify the actin cytoskeleton, preventing determinants and their adapters like Miranda from binding to the apical cortex. The tumor suppressor Lgl can bind directly to myosin II and negatively regulate filament formation and myosin activity. Lgl is uniformly distributed around the neuroblast cortex. However, at the apical cortex, Lgl is phosphorylated and inactivated by aPKC, releasing myosin II. Myosin II can form filaments and is active apically, excluding Miranda. Conversely, Lgl is active at the basal cortex where there is no aPKC. Lgl can then bind and inhibit myosin II, which in turn enables Miranda to localize to the basal cortex. (B) (Left) In the absence of Lgl, myosin II is active throughout the cell and can bind to the entire cortex, resulting in the exclusion of Miranda. Miranda accumulates at the spindle microtubules. (Right) When Lgl is activated throughout the cell (Lgl-3A), myosin II is inactivated and cannot bind to the cortex. As a result, Miranda spreads around the entire cortex. Myosin II, green; Lgl, blue; Miranda, red.

concentrates instead at the spindle microtubules. Therefore, active myosin is necessary from early development to organize the actin cytoskeleton, which is in turn required for Lgl and Miranda to localize to the cortex.

Myosin II is activated by phosphorylation of its regulatory light chain by Rho kinase (Amano et al., 1996; Kimura et al., 1996; Winter et al., 2001). We inactivated myosin at the time of neuroblast cell division by inhibition of Rho kinase. Myosin II no longer localizes at the apical neuroblast cortex but instead spreads into the cytoplasm, and basal protein localization is disrupted. Although F-actin and Lgl remain uniformly at the cortex, cell fate determinants are now found around the entire cell cortex, demonstrating that apical cortical myosin is required to confine determinants to the basal half of the cell. Inhibition of Rho kinase also blocks cytokinesis (Yasui et al., 1998), although the defect in basal protein localization is unlikely to be the consequence of mitotic arrest or a block in cytokinesis. First, basal protein localization is not disrupted in neuroblasts arrested in mitosis by colcemid treatment (Knoblich et al., 1995; Spana and Doe, 1995; Broadus and Doe, 1997). Second, mitosis occurs without cytokinesis in *pebble* mutants (Hime and Saint, 1992; Lehner, 1992), but the resultant polyploid neuroblasts still localize Numb and Prospero asymmetrically (Knoblich et al., 1995). Finally, the loss of asymmetry resulting from Rho kinase inhibition can be rescued by expression of a constitutively active form of the myosin II regulatory light chain (SqhE20E21). We conclude that myosin II is required to restrict cell fate determinants to the basal cortex.

Myosin II Is Asymmetrically Localized in Neuroblasts

Myosin II localizes to the apical cortex of metaphase neuroblasts. Why is myosin localization/activity asymmetric? Lgl binds myosin II heavy chain directly and inhibits myosin filament formation (Strand et al., 1994; Strand, 1998; De Lorenzo et al., 1999). This binding is regulated by phosphorylation of Lgl, which inhibits its interaction with myosin II in vitro (Kalmes et al., 1996). If Lgl negatively regulates myosin activity and localization, then myosin should be uniformly distributed in an *lgl* mutant. Indeed, we find that in *lgl*$^{1\ GLC}$ mutants myosin II no longer concentrates apically but is found uniformly around the cortex. Most Miranda protein is released from the cortex and binds microtubules, again suggesting that myosin excludes Miranda from the cortex.

We have shown that myosin II localizes to the entire cortex in *lgl* mutants and thereby prevents Miranda binding basally. In *Drosophila* neuroblasts in which Lgl levels are reduced (zygotic *lgl*^{1} mutants), Miranda is released from the cortex. Miranda localization can be rescued by simultaneously reducing the level of myosin II (*zip*^{1} zygotic mutants) (Ohshiro et al., 2000; Peng et al., 2000). Reducing the level of active myosin may restore the balance between the levels of Lgl and myosin, enabling the remaining myosin to concentrate apically.

Possible Mechanisms for Myosin II-Mediated Regulation of Protein Localization

How does myosin II restrict neuroblast proteins to the basal side of the cell cortex? Myosin II and Miranda occupy primarily opposite sides at the neuroblast cortex: myosin II is concentrated at the apical cortex while Miranda localizes as a basal crescent. As myosin II shifts to the cleavage furrow, Miranda is segregated into the forming GMC. The apical F-actin compartment may be modified by myosin II to exclude binding of basal proteins like Miranda. Active myosin II requires Rho kinase activity and depends on inactivation of Lgl at the apical cortex by aPKC (Betschinger et al., 2003). Ectopic expression of a nonphosphorylatable form of Lgl, in which the conserved aPKC-dependent phosphorylation sites are mutated from Serines to Alanines (Lgl-3A), results in mislocalization of Miranda around the neuroblast cortex (Betschinger et al., 2003). Our data supports a spatially regulated interaction between myosin II and Lgl. We have shown that myosin is apically localized in wild-type neuroblasts, corresponding to the domain in which Lgl is inactivated by aPKC. We further show that in *lgl* mutants, myosin is no longer restricted apically but localizes around the entire cell cortex. Conversely, when we express nonphosphorylatable Lgl in neuroblasts, myosin is inhibited throughout the cell and drops off the cortex. We propose that myosin II is activated and can form filaments at the apical cortex, where phosphorylated Lgl is inactive and unable to bind myosin II. Myosin may then modify the actin cytoskeleton to prevent the binding of Miranda (Figure 8A). At the basal cortex, in the absence of aPKC, Lgl is active and can bind and inhibit myosin. Myosin cannot form filaments, which are required for it to bind to the actin cortex. As a result, Miranda can bind to the basal cortex (Figure 8A).

At anaphase, myosin II moves to the equator and appears to "push" cell fate determinants into the daughter cell. This movement is regulated in an Lgl-independent fashion and occurs whether myosin is restricted to the apical cortex or is uniformly cortical (as in *lgl* mutants). Cortical myosin is essential, however, to efficiently segregate determinants into the GMC at telophase (telophase rescue; Peng et al., 2000; Cai et al., 2001). In neuroblasts expressing Lgl-3A, myosin II is cytoplasmic, and determinants are not partitioned to the daughter cell. Nonetheless, at telophase, myosin seems to be recruited from the cytoplasm, as it still accumulates to the cleavage furrow. We can thus define three separate steps of myosin regulation in neuroblasts. First, myosin forms an apical crescent. This is positively regulated by Rho kinase and negatively regulated by Lgl. Second, cortical myosin moves to the equator. This movement occurs independently of Lgl. Third, cortical and cytoplasmic myosin accumulates at the cleavage furrow, a step that is also Lgl independent. Rho Kinase activation seems to be important for all three steps of myosin II regulation. When Rho kinase is inhibited, myosin falls into the cytoplasm, and there is no cleavage furrow formation.

In conclusion, our results demonstrate that myosin II acts downstream of Lgl and the apical protein complex to regulate the segregation of cell fate determinants. Myosin II does not negatively regulate basal protein targeting, as has previously been suggested (Ohshiro et al., 2000; Peng et al., 2000) nor does it transport determinants directly. Instead, we propose that myosin II acts in a novel fashion, excluding determinants from the apical cortex and "pushing" them into the GMC at anaphase and telophase. Myosin II might modify the actin cytoskeleton to prevent determinants binding, although the actual structure formed and the physical change in the actin cytoskeleton remains to be determined.

Experimental Procedures

Drosophila Mutants and Transgenic Lines

The FLP-DFS system (Chou and Perrimon, 1992) was used to produce homozygous *sqh*[1] (Karess et al., 1991), *drok*[2] (Winter et al., 2001) and *lgl*[1] (Mechler et al., 1985) germline clones. *sqh*[1 GLC] embryos are either *sqh*[1]/*Y* or *sqh*[1]/+ (paternally rescued). No embryos were obtained from *drok*[2] germline clone females, possibly because *drok*[2], the only available mutant, is a strong loss-of-function allele (Winter et al., 2001). Drok may be essential for oogenesis.

sqh[AX3]; P [w, sqh-GFP42], in which sqh-GFP42 is the only source of myosin II RLC, and sqhE20E21 have been described previously (Royou et al., 2002). Expression of pUAS-Lgl-3A (Betschinger et al., 2003) was driven by the GAL4 driver V32 (Hacker and Perrimon, 1998).

To express GFP-Zipper, we generated pUAST-mGFP6-ZipperWT. The N terminus of Zipper was PCR amplified from pBS-Zipper (a kind gift from Dr. Dan Kiehart) using primers CBP55/CBP56 (Phelps, 2000) to introduce a KpnI site and maintain the NruI site. Next, pBS-Zipper was digested with KpnI and NruI to excise the N-terminal fragment of Zipper. The PCR-engineered KpnI-Zipper-N-terminus-NruI fragment was then inserted into the KpnI/NruI cut pBS-Zipper-backbone. mGFP6 (Schuldt et al., 1998) was PCR amplified from pmGFP6 (a kind gift from Dr. Jim Haseloff) with primers CBP57/CBP58 (Phelps, 2000) to introduce EcoRI and KpnI sites. mGFP6 was inserted into KpnI cut pBS-engineered Zipper. An EcoRI-mGFP6-ZipperWT-NotI cassette was then inserted into pUAST (Brand and Perrimon, 1993) to produce pUAST-mGFP6-ZipperWT. Transgenic flies were generated as described previously (Brand and Perrimon, 1993), except that DNA was prepared using a Qiagen midiprep kit, and embryos were dechorionated with bleach rather than by hand. UAS-mGFP6-ZipperWT expression was driven by the GAL4 driver V37 (Hacker and Perrimon, 1998). *zip*[1] homozygous mutants (Young et al., 1993) were rescued by expression of pUAS-mGFP6-ZipperWT driven by *daughterless*GAL4 (Wodarz et al., 1995).

Drug Treatment

yw (Bloomington) embryos were used for injection of Rho-kinase inhibitor Y-27632 (17 mg/ml in water; TOCRIS). Embryos were injected laterally at stage 9/10 and allowed to develop at 18°C for 30 min.

Immunohistochemistry

Fixation and immunohistochemistry were performed as described in (Bossing et al., 2002). In brief, embryos were fixed in 20% formaldehyde in PBS, 0.3% Triton X-100 (PBT). After fixation, embryos were washed with PBS, and the vitelline membranes were removed manually. After washes in methanol, embryos were rehydrated in PBT and incubated in primary antibody. 80% ethanol was used in place of methanol when staining for myosin II, F-actin, and Lgl. Primary antibodies were diluted in PBT and used at the following concentrations: rabbit anti-Miranda, 1:1500 (Ohshiro et al., 2000); rabbit anti-Miranda A96C, 1:1000 (Shen et al., 1997); mouse anti-Miranda, 1:3 (Ohshiro et al., 2000); rabbit anti-Numb, 1:1000 (Ohshiro et al., 2000); mouse anti-Prospero MR1A, 1:2 (Spana and Doe, 1995); rabbit anti-Bazooka 1:500 (Wodarz et al., 1999); rabbit anti-myosin II heavy chain (Zipper); 1:750 (Foe et al., 2000); rabbit anti-Lgl-N, 1:100 (Ohshiro et al., 2000); mouse anti-α-Tubulin, 1:500 (Sigma); rabbit anti-β-Galactosidase, 1:1000 (Cappel); rabbit anti-GFP 290, 1:1000 (Abcam). Secondary antibodies coupled to Alexa[488nm] and Alexa[568nm] (Molecular Probes) were diluted in PBT and used at 1:200. DNA was stained with TOTO-3 (Molecular Probes, 1:5000 in PBT). F-actin was labeled with 20 nM Phalloidin-Alexa[568nm] (Molecular

Probes). Embryos were mounted in Vectashield (Vector Laboratories) and visualized by confocal microscopy.

Confocal Microscopy

Images of fixed embryos were collected using a BioRad MRC1024 scan head on a Nikon E800 microscope. Images were imported into Adobe Photoshop and assembled in Adobe Illustrator.

Live Imaging

Embryos were mounted on air-permeable Teflon membranes in Voltalef oil (ELF Atochem) as described previously (Brand, 1999). GFP time-lapse series were collected using a Radiance 2000 scan head on a Nikon E800 microscope. Images were imported into Adobe Photoshop and assembled in Adobe Illustrator or imported into Adobe Premiere and converted to QuickTime movies.

Acknowledgments

For generously providing DNA constructs, antibodies, and *Drosophila* lines, we thank Bill Chia, Catherine Davidson, Chris Doe, Jim Haseloff, Roger Karess, Dan Kiehart, Jurgen Knoblich, Eli Knust, Liqun Luo, Fumio Matsuzaki, Christine Miller, Daniel St. Johnston, Andreas Wodarz, and Yuh Nung Yan. For helpful discussions and/or comments on the manuscript, we thank Yohanns Bellaiche, Torsten Bossing, Adrian Carr, Karin Edoff, David Elliott, Roger Karess, Peter van Roessel, Francois Schweisguth, and Guy Tanentzapf. C.S.B. was supported by a studentship from the Fundação para a Ciência e Tecnologia, Portugal. CBP was supported by a Wellcome Trust Prize studentship. This work was funded by a Wellcome Trust Senior Fellowship to A.H.B.

Received: August 20, 2003
Revised: October 17, 2003
Accepted: October 23, 2003
Published: December 8, 2003

References

Amano, M., Ito, M., Kimura, K., Fukata, Y., Chihara, K., Nakano, T., Matsuura, Y., and Kaibuchi, K. (1996). Phosphorylation and activation of myosin by Rho-associated kinase (Rho- kinase). J. Biol. Chem. *271*, 20246–20249.

Betschinger, J., Mechtler, K., and Knoblich, J.A. (2003). The Par complex directs asymmetric cell division by phosphorylating the cytoskeletal protein Lgl. Nature *422*, 326–330.

Bossing, T., Barros, C.S., and Brand, A.H. (2002). Rapid tissue-specific expression assay in living embryos. Genesis *34*, 123–126.

Brand, A. (1999). GFP as a cell and developmental marker in the *Drosophila* nervous system. In Green Fluorescent Proteins, K.F. Sullivan and S.A. Kay, eds. (La Jolla, CA: Academic Press), pp. 165–181.

Brand, A.H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development *118*, 401–415.

Bresnick, A.R. (1999). Molecular mechanisms of nonmuscle myosin-II regulation. Curr. Opin. Cell Biol. *11*, 26–33.

Broadus, J., and Doe, C.Q. (1997). Extrinsic cues, intrinsic cues and microfilaments regulate asymmetric localization in *Drosophila* neuroblasts. Curr. Biol. *7*, 827–835.

Cai, Y., Chia, W., and Yang, X. (2001). A family of snail-related zinc finger proteins regulates two distinct and parallel mechanisms that mediate Drosophila neuroblast asymmetric divisions. EMBO J. *20*, 1704–1714.

Cayouette, M., and Raff, M. (2002). Asymmetric segregation of Numb: a mechanism for neural specification from *Drosophila* to mammals. Nat. Neurosci. *5*, 1265–1269.

Cheung, A., Dantzig, J.A., Hollingworth, S., Baylor, S.M., Goldman, Y.E., Mitchison, T.J., and Straight, A.F. (2002). A small-molecule inhibitor of skeletal muscle myosin II. Nat. Cell Biol. *4*, 83–88.

Chou, T.-B., and Perrimon, N. (1992). Use of a Yeast site-specific recombinase to produce female germline chimeras in *Drosophila*. Genetics *131*, 643–653.

Cuenca, A.A., Schetter, A., Aceto, D., Kemphues, K., and Seydoux, G. (2003). Polarization of the *C. elegans* zygote proceeds via distinct establishment and maintenance phases. Development *130*, 1255–1265.

De Lorenzo, C., Mechler, B.M., and Bryant, P.J. (1999). What is Drosophila telling us about cancer? Cancer Metastasis Rev. *18*, 295–311.

Doe, C.Q., Chu-LaGraff, Q., Wright, D.M., and Scott, M.P. (1991). The *prospero* gene specifies cell fates in the *Drosophila* central nervous system. Cell *65*, 451–465.

Edwards, K.A., and Kiehart, D.P. (1996). Drosophila nonmuscle myosin II has multiple essential roles in imaginal disc and egg chamber morphogenesis. Development *122*, 1499–1511.

Foe, V.E., Field, C.M., and Odell, G.M. (2000). Microtubules and mitotic cycle phase modulate spatiotemporal distributions of F-actin and myosin II in Drosophila syncytial blastoderm embryos. Development *127*, 1767–1787.

Gateff, E. (1978). Malignant neoplasms of genetic origin in *Drosophila melanogaster*. Science *200*, 1448–1459.

Guo, S., and Kemphues, K.J. (1996). A non-muscle myosin is required for embryonic polarity in *Caenorhabditis elegans*. Nature *382*, 455–458.

Hacker, U., and Perrimon, N. (1998). *DRhoGEF2* encodes a member of the Dbl family of oncogenes and controls cell shape changes during gastrulation in *Drosophila*. Genes Dev. *12*, 274–284.

Hime, G., and Saint, R. (1992). Zygotic expression of the pebble locus is required for cytokinesis during the postblastoderm mitoses of Drosophila. Development *114*, 165–171.

Ikeshima-Kataoka, H., Skeath, J.B., Nabeshima, Y., Doe, C.Q., and Matsuzaki, F. (1997). Miranda directs Prospero to a daughter cell during *Drosophila* asymmetric divisions. Nature *390*, 625–629.

Jan, Y.N., and Jan, L.Y. (2001). Asymmetric cell division in the Drosophila nervous system. Nat. Rev. Neurosci. *2*, 772–779.

Jordan, P., and Karess, R. (1997). Myosin light chain-activating phosphorylation sites are required for oogenesis in Drosophila. J. Cell Biol. *139*, 1805–1819.

Kalmes, A., Merdes, G., Neumann, B., Strand, D., and Mechler, B.M. (1996). A serine-kinase associated with the p127-l(2)gl tumour suppressor of Drosophila may regulate the binding of p127 to nonmuscle myosin II heavy chain and the attachment of p127 to the plasma membrane. J. Cell Sci. *109*, 1359–1368.

Karess, R.E., Chang, X.J., Edwards, K.A., Kulkarni, S., Aguilera, I., and Kiehart, D.P. (1991). The regulatory light chain of nonmuscle myosin is encoded by spaghetti-squash, a gene required for cytokinesis in *Drosophila*. Cell *65*, 1177–1189.

Kawano, Y., Fukata, Y., Oshiro, N., Amano, M., Nakamura, T., Ito, M., Matsumura, F., Inagaki, M., and Kaibuchi, K. (1999). Phosphorylation of myosin-binding subunit (MBS) of myosin phosphatase by Rho-kinase in vivo. J. Cell Biol. *147*, 1023–1038.

Kiehart, D.P., Lutz, M.S., Chan, D., Ketchum, A.S., Laymon, R.A., Nguyen, B., and Goldstein, L.S. (1989). Identification of the gene for fly non-muscle myosin heavy chain: Drosophila myosin heavy chains are encoded by a gene family. EMBO J. *8*, 913–922.

Kimura, K., Ito, M., Amano, M., Chihara, K., Fukata, Y., Nakafuku, M., Yamamori, B., Feng, J., Nakano, T., Okawa, K., et al. (1996). Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). Science *273*, 245–248.

Knoblich, J.A. (2001). Asymmetric cell division during animal development. Nat. Rev. Mol. Cell Biol. *2*, 11–20.

Knoblich, J.A., Jan, L.Y., and Jan, Y.N. (1995). Asymmetric segregation of Numb and Prospero during cell division. Nature *377*, 624–630.

Knoblich, J.A., Jan, L.Y., and Jan, Y.N. (1997). The N-terminus of the *Drosophila* Numb protein directs membrane association and actin-dependent asymmetric localization. Proc. Natl. Acad. Sci. USA *94*, 13005–13010.

Kraut, R., and Campos-Ortega, J.A. (1996). *inscuteable*, a neural precursor gene of *Drosophila*, encodes a candidate for a cytoskeletal adapter protein. Dev. Biol. *174*, 65–81.

Kraut, R., Chia, W., Jan, L.Y., Jan, Y.N., and Knoblich, J.A. (1996).

Role of *inscuteable* in orienting asymmetric cell divisions in *Drosophila*. Nature *383*, 50–55.

Kuchinke, U., Grawe, F., and Knust, E. (1998). Control of spindle orientation in *Drosophila* by the Par-3-related PDZ-domain protein Bazooka. Curr. Biol. *8*, 1357–1365.

Lehner, C.F. (1992). The pebble gene is required for cytokinesis in Drosophila. J. Cell Sci. *103*, 1021–1030.

Lu, B., Ackerman, L., Jan, L.Y., and Jan, Y.N. (1999). Modes of protein movement that lead to the asymmetric localization of partner of Numb during *Drosophila* neuroblast division. Mol. Cell *4*, 883–891.

Lu, B., Jan, L., and Jan, Y.N. (2000). Control of cell divisions in the nervous system: symmetry and asymmetry. Annu. Rev. Neurosci. *23*, 531–556.

Matsumura, F., Totsukawa, G., Yamakita, Y., and Yamashiro, S. (2001). Role of myosin light chain phosphorylation in the regulation of cytokinesis. Cell Struct. Funct. *26*, 639–644.

Mechler, B.M., McGinnis, W., and Gehring, W.J. (1985). Molecular cloning of lethal(2)giant larvae, a recessive oncogene of Drosophila melanogaster. EMBO J. *4*, 1551–1557.

Narumiya, S., Ishizaki, T., and Uehata, M. (2000). Use and properties of ROCK-specific inhibitor Y-27632. Methods Enzymol. *325*, 273–284.

Ohshiro, T., Yagami, T., Zhang, C., and Matsuzaki, F. (2000). Role of cortical tumour-suppressor proteins in asymmetric division of Drosophila neuroblast. Nature *408*, 593–596.

Parmentier, M.L., Woods, D., Greig, S., Phan, P.G., Radovic, A., Bryant, P., and O'Kane, C.J. (2000). Rapsynoid/partner of inscuteable controls asymmetric division of larval neuroblasts in Drosophila. J. Neurosci. *20*, RC84.

Peng, C.Y., Manning, L., Albertson, R., and Doe, C.Q. (2000). The tumour-suppressor genes lgl and dlg regulate basal protein targeting in Drosophila neuroblasts. Nature *408*, 596–600.

Petrisch, C., Tavosanis, G., Turck, C.W., Jan, L.Y., and Jan, Y.N. (2003). The *Drosophila* myosin VI jaguar is required for basal protein targeting and correct spindle orientation in mitotic neuroblasts. Dev. Cell *4*, 273–281.

Petronczki, M., and Knoblich, J.A. (2001). DmPAR-6 directs epithelial polarity and asymmetric cell division of neuroblasts in Drosophila. Nat. Cell Biol. *3*, 43–49.

Phelps, C.B. (2000). Myosin-II regulates the segregation of cell fate determinants in the *Drosophila* CNS. PhD Thesis, University of Cambridge, Cambridge, United Kingdom.

Plant, P.J., Fawcett, J.P., Lin, D.C., Holdorf, A.D., Binns, K., Kulkarni, S., and Pawson, T. (2003). A polarity complex of mPar-6 and atypical PKC binds, phosphorylates and regulates mammalian Lgl. Nat. Cell Biol. *5*, 301–308.

Rhyu, M.S., Jan, L.Y., and Jan, Y.N. (1994). Asymmetric distribution of Numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells. Cell *76*, 477–491.

Royou, A., Sullivan, W., and Karess, R. (2002). Cortical recruitment of nonmuscle myosin II in early syncytial Drosophila embryos: its role in nuclear axial expansion and its regulation by Cdc2 activity. J. Cell Biol. *158*, 127–137.

Ryan, K.R., and Shapiro, L. (2003). Temporal and spatial regulation in prokaryotic cell cycle progression and development. Annu. Rev. Biochem.

Schaefer, M., Shevchenko, A., and Knoblich, J.A. (2000). A protein complex containing Inscuteable and the Galpha-binding protein Pins orients asymmetric cell divisions in *Drosophila*. Curr. Biol. *10*, 353–362.

Schaefer, M., Petronczki, M., Dorner, D., Forte, M., and Knoblich, J.A. (2001). Heterotrimeric G proteins direct two modes of asymmetric cell division in the *Drosophila* nervous system. Cell *107*, 183–194.

Schober, M., Schaefer, M., and Knoblich, J.A. (1999). Bazooka recruits Inscuteable to orient asymmetric cell divisions in Drosophila neuroblasts. Nature *402*, 548–551.

Schuldt, A., Adams, J.H.J., Davidson, C.M., Micklem, D.R., Haseloff, J., St Johnston, D., and Brand, A.H. (1998). Miranda mediates asymmetric protein and RNA localisation in the developing nervous system. Genes Dev. *12*, 1847–1857.

Severson, A.F., and Bowerman, B. (2003). Myosin and the PAR proteins polarise microfilament-dependent forces that shape and position spindles in *Caenorhabditis elegans*. J. Cell Biol. *161*, 21–26.

Shelton, C.A., Carter, J.C., Ellis, G.C., and Bowerman, B. (1999). The nonmuscle myosin regulatory light chain gene mlc-4 is required for cytokinesis, anterior-posterior polarity, and body morphology during Caenorhabditis elegans embryogenesis. J. Cell Biol. *146*, 439–451.

Shen, C.P., Jan, L.Y., and Jan, Y.N. (1997). Miranda is required for the asymmetric localisation of Prospero during mitosis in *Drosophila*. Cell *90*, 449–458.

Shen, C.P., Knoblich, J.A., Chan, Y.M., Jiang, M.M., Jan, L.Y., and Jan, Y.N. (1998). Miranda as a multidomain adapter linking apically localized Inscuteable and basally localized Staufen and Prospero during asymmetric cell division in Drosophila. Genes Dev. *12*, 1837–1846.

Spana, E.P., and Doe, C.Q. (1995). The prospero transcription factor is asymmetrically localized to the cell cortex during neuroblast mitosis in *Drosophila*. Development *121*, 3187–3195.

Straight, A.F., Cheung, A., Limouze, J., Chen, I., Westwood, N.J., Sellers, J.R., and Mitchison, T.J. (2003). Dissecting temporal and spatial control of cytokinesis with a myosin II inhibitor. Science *299*, 1743–1747.

Strand, D. (1998). The tumour suppresor l(2)gl; a myosin II-binding protein family. In G Proteins, Cytoskeleton and Cancer, H. Murata, and K. Kohama, eds. (Georgetown,TX: R.G. Landes Company), 61–78.

Strand, D., Jakobs, R., Merdes, G., Neumann, B., Kalmes, A., Heid, H.W., Husmann, I., and Mechler, B.M. (1994). The Drosophila lethal(2)giant larvae tumor suppressor protein forms homo-oligomers and is associated with nonmuscle myosin II heavy chain. J. Cell Biol. *127*, 1361–1373.

Tan, J.L., Ravid, S., and Spudich, J.A. (1992). Control of nonmuscle myosins by phosphorylation. Annu. Rev. Biochem. *61*, 721–759.

Uemura, T., Shepherd, S., Ackerman, L., Jan, L.Y., and Jan, Y.N. (1989). *numb,* a gene required in determination of cell fate during sensory organ formation in *Drosophila* embryos. Cell *58*, 349–360.

Vaessin, H., Grell, E., Wolff, E., Bier, E., Jan, L.Y., and Jan, Y.N. (1991). *prospero* is expressed in neuronal precursors and encodes a nuclear protein that is involved in the control of axonal outgrowth in *Drosophila*. Cell *67*, 941–953.

Winter, C.G., Wang, B., Ballew, A., Royou, A., Karess, R., Axelrod, J.D., and Luo, L. (2001). *Drosophila* Rho-associated kinase (Drok) links Frizzled-mediated planar cell polarity signaling to the actin cytoskeleton. Cell *105*, 81–91.

Wodarz, A., Hinz, U., Engelbert, M., and Knust, E. (1995). Expression of crumbs confers apical character on plasma membrane domains of ectodermal epithelia of *Drosophila*. Cell *82*, 67–76.

Wodarz, A., Ramrath, A., Kuchinke, U., and Knust, E. (1999). Bazooka provides an apical cue for Inscuteable localization in Drosophila neuroblasts. Nature *402*, 544–547.

Wodarz, A., Ramrath, A., Grim, A., and Knust, E. (2000). *Drosophila* atypical protein kinase C associates with Bazooka and controls polarity of epithelia and neuroblasts. J. Cell Biol. *150*, 1361–1374.

Woods, D.F., and Bryant, P.J. (1991). The discs-large tumor suppressor gene of *Drosophila* encodes a guanylate kinase homologue localized at septate junctions. Cell *66*, 451–464.

Yarrow, J.C., Lechler, T., Li, R., and Mitchison, T.J. (2003). Rapid delocalisation of actin leading edge components with BDM treatment. BMC Cell Biol. *4*, 5. Published online June 3, 2003.

Yasui, Y., Amano, M., Nagata, K., Inagaki, N., Nakamura, H., Saya, H., Kaibuchi, K., and Inagaki, M. (1998). Roles of Rho-associated kinase in cytokinesis; mutations in Rho-associated kinase phosphorylation sites impair cytokinetic segregation of glial filaments. J. Cell Biol. *143*, 1249–1258.

Young, P.E., Richman, A.M., Ketchum, A.S., and Kiehart, D.P. (1993). Morphogenesis in Drosophila requires nonmuscle myosin heavy chain function. Genes Dev. *7*, 29–41.

Yu, F., Morin, X., Cai, Y., Yang, X., and Chia, W. (2000). Analysis of partner of inscuteable, a novel player of *Drosophila* asymmetric divisions, reveals two distinct steps in inscuteable apical localization. Cell *100*, 399–409.