MYOSIN DYNAMICS IN *DROSOPHILA* NEUROBLASTS LEAD TO ASYMMETRIC CYTOKINESIS

by

MARISA SUE CONNELL

A DISSERTATION

Presented to the Department of Biology
and the Graduate School of the University of Oregon
in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy

March 2013

DISSERTATION APPROVAL PAGE

Student: Marisa Sue Connell

Title: Myosin Dynamics in *Drosophila* Neuroblasts Lead to Asymmetric Cytokinesis

This dissertation has been accepted and approved in partial fulfillment of the requirements for the Doctor of Philosophy degree in the Department of Biology by:

Dr. Karen Guillemin	Chair
Dr. Kenneth Prehoda	Advisor
Dr. Chris Doe	Member
Dr. Bruce Bowerman	Member
Dr. Brad Nolen	Outside Member

and

Dr. Kimberly Andrews Espy	Vice President for Research & Innovation/ Dean of the Graduate School

Original approval signatures are on file with the University of Oregon Graduate School.

Degree awarded March 2013

DISSERTATION ABSTRACT

Marisa Sue Connell

Doctor of Philosophy

Department of Biology

March 2013

Title: Myosin Dynamics in *Drosophila* Neuroblasts Lead to Asymmetric Cytokinesis

Cells divide to create two daughter cells through cytokinesis. Daughter cells of different sizes are created by shifting the position of the cleavage furrow. The cleavage furrow forms at the position of the metaphase plate so in asymmetric cytokinesis the spindle is shifted towards one pole. Unlike most systems, *Drosophila* neuroblasts have a centrally localized metaphase plate but divide asymmetrically. *Drosophila* neuroblasts divide asymmetrically due to the presence of a polarized myosin domain at the basal pole during mitosis. I investigated the mechanism by which the basal myosin domain produces asymmetric cytokinesis and the pathway regulating this domain.

We tested several mechanisms by which the basal myosin domain could lead to asymmetric cytokinesis. Based on surface area and volume measurements, I demonstrated that asymmetric addition of new membrane is not involved. I determined that neuroblasts exhibit asymmetric cortical extension during anaphase with the apical pole extending 2-3 times more than the basal pole. Mutants that lose basal myosin extend equally at both poles supporting this model. Mutants that retain apical myosin exhibited symmetric cortical extension but still divided

asymmetrically, demonstrating that asymmetric cortical extension is not required for asymmetric cytokinesis. Observations of the mitotic spindle show that the cleavage furrow forms at a position biased towards the basal pole when compared to the position of the metaphase plate even though this position is still equidistant between the centrosomes. I observed that midzone components shift basally in a basal domain dependent manner suggesting that contraction of the basal domain leads to new microtubule-cortex interactions at a position away from the spindle midzone.

I demonstrated that the basal domain is regulated by the heterotrimeric G protein, Gβ13F, which is activated by Pins. In Gβ mutants, the localization of all basal components (myosin, anillin, and pavarotti) is lost and the cells divide symmetrically. Although the basal domain is contiguous with equatorial myosin, it is not regulated by the same pathway and photobleaching experiments indicate that they exhibit different behaviors during anaphase suggesting a difference in temporal regulation.

This dissertation includes previously published coauthored material.

CURRICULUM VITAE

NAME OF AUTHOR: Marisa Sue Connell

GRADUATE AND UNDERGRADUATE SCHOOLS ATTENDED:

University of Oregon, Eugene, Oregon
Portland State University, Portland, Oregon
Reed College, Portland, Oregon
University of California at Berkeley, Berkeley, California

DEGREES AWARDED:

Doctor of Philosophy in Biology, 2013, University of Oregon
Bachelor of Arts in Biochemistry & Molecular Biology, 2004, Reed College

AREAS OF SPECIAL INTEREST:

Cell Biology
Cell Division

PROFESSIONAL EXPERIENCE:

Graduate Research Fellow, Department of Biology, Dr. Kenneth Prehoda, University of Oregon, 2007-2013

Graduate Teaching Fellow, Department of Biology, Department of Chemistry, University of Oregon, 2007-2013

Undergraduate Researcher, Dr. Michael Bartlett, Portland State University, 2006

Undergraduate Researcher, Dr. Janis Shampay, Reed College, 2003-2004

GRANTS, AWARDS AND HONORS:

National Institutes of Health Genetics Training Grant Appointee, University of Oregon, 2008-2011

PUBLICATIONS:

Connell, M., Cabernard, C., Ricketson, D., Doe, C.Q., and Prehoda, K.E. (2011) Asymmetric cortical extension shifts cleavage furrow position in *Drosophila* neuroblasts. *Mol Biol Cell*, 22:4220-4226.

ACKNOWLEDGEMENTS

I wish to express sincere gratitude to my advisor Dr. Ken Prehoda for his guidance throughout my graduate career. His mentorship was essential in preparing me for my future career in science by providing me with the experimental and critical thinking skills I need. I would like to thank the members of my committee for their input into my research project and their professional guidance. In addition, I would like to thank the members of my lab for their helpful suggestions and discussions about my project as well as their support during the times when experiments were providing me with frustration. I also want to thank members of the Doe lab for sharing fly stocks and reagents. Specifically, I want to thank Keiko Hirono for constructs and microscope training and Clemens Cabernard for his collaboration on Chapter II of this thesis, microscope training, and for sharing fly stocks. This work was supported by NIH grant ROI 068032 awarded to K. Prehoda and NIH Genetics Training Grant to M. Connell.

TABLE OF CONTENTS

LIST OF FIGURES

Figure Page

LIST OF TABLES

Table Page

CHAPTER I

INTRODUCTION

During development cells often need to divide to create cells that differ in size, protein content, or both in order to produce all the different cell types necessary to form an organism. Different sized daughter cells are created in a process called asymmetric cytokinesis where the complex that cleaves the mother cell into two cells is biased towards one pole of the cell rather than being centrally localized. Our model system, the *Drosophila* neuroblast divides asymmetrically to produce a self-renewing neuroblast and a differentiating ganglion mother cell (GMC). *Drosophila* neuroblasts have a unique mechanism for producing asymmetric cytokinesis and this dissertation investigates the mechanism and the pathway regulating it.

The process of cell division in called mitosis and mitosis only accounts for a small portion of the cell cycle, the bulk of which is spent growing and synthesizing new DNA in preparation for mitosis. Mitosis is divided into different stages. In the first several phases, the chromosomes condense (prophase), the nuclear envelope breaks down (prometaphase), and the chromosomes align at the metaphase plate (metaphase). At this point, the cell is prepared to separate into two daughter cells. The mitotic spindle pulls apart and separates the chromosomes (anaphase), the nuclei reform (telophase) and there is a contraction of the cortex to cleave the cell into two daughter cells (cytokinesis). The region of the cortex that contracts is known as the cleavage furrow and the position of this domain is what determines the size of the two daughter cells.

The contractile force at the cleavage furrow is due to the formation of a contractile ring at the equator of the cell. The key component of this ring is non-muscle myosin II (referred to as myosin hereafter). Myosin is activated by phosphorylation of the regulatory light chain (Spaghetti Squash (Sqh) in *Drosophila*) and then forms bipolar filaments that interact with actin to produce the contractile forces needed at the cleavage furrow.

Positioning the Cleavage Furrow

The symmetry of cytokinesis is determined based on the position of the cleavage furrow which is in turn dictated by the position of the mitotic spindle (Figure 1.1) (Oliferenko et al., 2009; von Dassow, 2009). The cleavage furrow forms at a position that is half-way between the two poles of the mitotic spindle and this correlates to the spindle midzone. The spindle midzone contains protein complexes that organize the cleavage furrow and resolve the two cells once cytokinesis is finished.

There have been many models proposed as to how the cleavage furrow is positioned by the mitotic spindle (reviewed in Burgess and Chang, 2005). The equatorial stimulation model proposes that the mitotic spindle imparts an activating signal to promote furrow formation while the polar relaxation model suggests that astral microtubules relay signals to the polar cortex that locally inhibits cortical tension. The spindle midzone model proposes that the spindle midzone induces the furrow while the kinetochore signaling model posits that the kinetochore microtubules provide the signal.

Recent work in *C. elegans* embryos has shed some light on how the mitotic spindle is able to specify the position of the cleavage furrow and it was found

that positioning of the furrow is regulated by several pathways (Bringmann and Hyman, 2005). These experiments severed the spindle so that the position of the spindle midzone and the point halfway between the asters were no longer the same position. The embryos formed two furrows, one at the position of the spindle midzone and one at the position halfway between the two asters. These overlapping mechanisms work together to form the tight localization of the cleavage furrow rather than a broad domain (Bement et al., 2006).

In symmetric cytokinesis, the mitotic spindle localizes with the metaphase plate/spindle midzone in the center of the cell This leads to the formation of the cleavage furrow at the center of the cell and the production of daughter cells of similar sizes.

In many cases of asymmetric cytokinesis, such as in the first division of *C. elegans* embryos, a similar mechanism is used to produce asymmetric cytokinesis. The key difference between asymmetric and symmetric cytokinesis in these cases is that the mitotic spindle is not located centrally in the cell and is instead biased towards one pole (Glotzer, 2004). This causes the spindle midzone/metaphase plate to be localized closer to one pole and the cleavage furrow will subsequently form closer to one pole creating a larger daughter cell and a smaller daughter cell. Manipulations that affect the localization of the mitotic spindle in *C. elegans* embryos affect the asymmetry of the divisions. *C. elegans* embryos that are mutant for Gα have centrally localized spindles and the embryo divides to create two equal-sized daughter cells (Gotta and Ahringer, 2001).

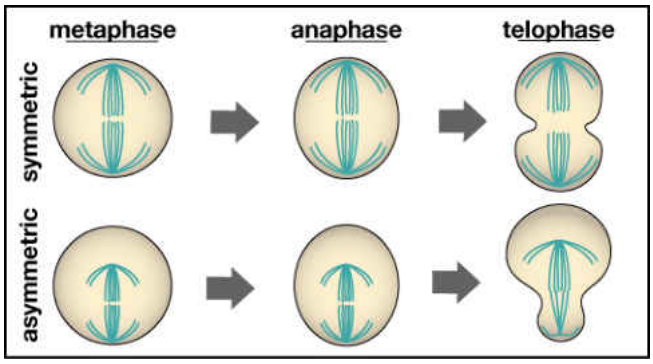

FIGURE 1.1. The mitotic spindle dictates the position of the cleavage furrow. In symmetrically dividing cells (top), the mitotic spindle localizes centrally leading to a centrally localized furrow. In most cases of asymmetric divisions (bottom), the spindle is biased towards one pole, leading to the furrow also being biased towards one pole.

Asymmetric Cytokinesis in *Drosophila* Neuroblasts

Our model system, the *Drosophila* neuroblast, undergoes asymmetric cytokinesis to produce daughter cells of different sizes and protein content (Knoblich, 2008). The larger daughter cell retains the neuroblast identity and continues to divide and produce the cells of the nervous system. The smaller daughter cell differentiates into a ganglion mother cell (GMC), which divides once more to give rise to neurons or glia. The different protein content is responsible for the change in cell fate in the daughter cells after mitosis but importance of asymmetric cytokinesis is not fully known. It is hypothesized that the neuroblasts need to retain as much size as possible in order to divide again quickly as cells must reach a particular size threshold before they divide again (Joregensen and Tyers, 2004). In fact, the daughter neuroblasts retain between 80-90% of their size after cytokinesis.

Drosophila neuroblasts divide asymmetrically but they differ from other cases of asymmetric cytokinesis in that they divide asymmetrically even though they have a centrally localized mitotic spindle. To account for this discrepancy previous

research focused on asymmetric characteristics of the mitotic spindle: (1) the apical centrosome is larger; (2) the apical astral microtubules grow more robustly; and (3) the apical centrosome is farther from the cortex (Fuse et al., 2003). These conclusions were made from observation of Gβ13F mutant neuroblasts which lose spindle asymmetry and produce daughter cells of equal sizes (Fuse et al., 2003). This model is not supported by *asterless* (*asl*) mutants which have symmetric spindles lacking the astral microtubules and still divide asymmetrically (Bonaccorsi et al., 1999). This suggested that the asymmetry of the spindle is not the factor that leads to asymmetric cytokinesis in *Drosophila* neuroblasts.

Recently, it has been reported that certain asymmetrically dividing cells, *Drosophila* neuroblasts and *C. elegans* Q neuroblasts, there is a polarized myosin domain that is required for asymmetric divisions (Figure 1.2) (Cabernard et al., 2010; Ou et al., 2010). Loss of the polarized myosin domain leads to symmetric divisions in both systems. The polarized myosin domain in *Drosophila* neuroblasts has been shown to be regulated by Pins (Cabernard et al., 2010) while the polarized myosin domain in *C. elegans* is regulated by the serine-threonine kinase pig-1 (Ou et al., 2010). Pig-1 does not have a *Drosophila* homolog suggesting these two domains may be regulated differently. In addition, in *Drosophila* it was discovered that the cleavage furrow components, Anillin (Scraps in *Drosophila*) and Pavarotti (Pav) also localize to the basal domain (Cabernard et al., 2010). This suggests that perhaps the polarized myosin domain in *Drosophila* could be regulated similarly to the cleavage furrow during mitosis and the pathways that regulate myosin during mitosis are described in the following section.

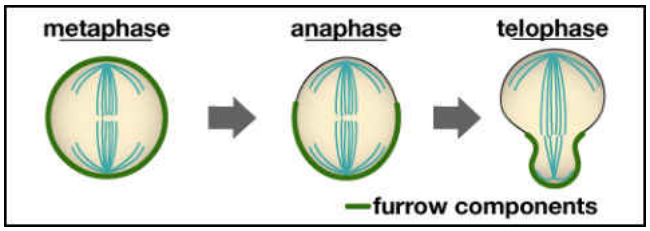

FIGURE 1.2. *Drosophila* neuroblasts divide asymmetrically because of a polarized myosin domain which localizes to the basal pole. The furrow components, myosin, anillin, and pavarotti, localize to the basal cortex during anaphase. In mutants lacking the basal domain, neuroblasts divide symmetrically showing this domain is required for asymmetric division in *Drosophila* neuroblasts.

Regulation of Myosin in Mitosis

Myosin acts in many different contexts in the cell, such as cell motility, cytokinesis, and regulation of cell shape, and is regulated by many different pathways. In general, myosin exists as a self-inhibited monomer composed of the myosin heavy chain, the regulatory light chain, and the essential light chain. Phosphorylation of the regulatory light chain (Spaghetti Squash (Sqh) in *Drosophila*) causes loss of inhibition and allows the formation of bipolar myosin filaments. Sqh is phosphorylated by many different kinases but is mainly regulated by Rho kinase (Rok) during mitosis. The pathways that regulate cortical dynamics of myosin during mitosis have been thoroughly investigated and are described in the following sections.

Regulation of myosin at the cell cortex

In mitosis, myosin serves several different functions and at the onset of mitosis it localizes to the cortex from the cytoplasm. The cortical localization of myosin is due to Rho GTPase (Rho) activation of Rho kinase and the subsequent phosphorylation of Sqh to induce filament formation. Myosin localizes to the cortex

during prophase and works together with moesin to cause rounding of the cell so that it is spherical in shape prior to the separation of the chromosomes (Kunda et al., 2008). Establishment of the rounded cell shape is important for the cell to localize the spindle to its proper location. Photobleaching experiments have shown that during this time period, myosin is very dynamic with a high rate of exchange between the cortical population of myosin and the cytoplasmic population (Uehara et al., 2010).

Once the cell enters anaphase and the mitotic spindle starts to pull apart, myosin is lost from the polar regions of the cortex and is only retained at the equatorial region. There have been many models proposed for how this myosin localization pattern forms (discussed previously) but the regulatory pathway for the loss of polar myosin is unknown. The exceptions to this patterning are *Drosophila* neuroblasts and *C elegans* Q neuroblasts that lose myosin only at one pole while the myosin that remains forms the polarized myosin domain that is required for asymmetric cell division.

Regulation of myosin at the cleavage furrow

The equatorial myosin remaining after anaphase onset is organized with actin into a contractile ring that provides the constrictive force during cytokinesis. The pathways that regulate the formation of the contractile ring are very well studied and involve several converging pathways (Figure 1.3). Once the contractile ring is established it must also form connections to the cell's plasma membrane and the mitotic spindle so the cell is properly able to undergo cytokinesis.

The most upstream components of this pathway are the mitotic kinases, Aurora B and Polo, which serve multiple functions during mitosis in addition to

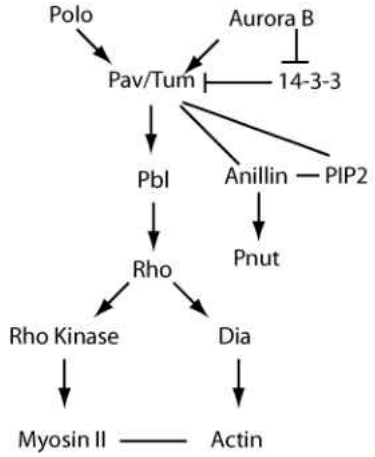

FIGURE 1.3. Schematic of the pathway regulating cleavage furrow formation during mitosis.

their role in cleavage furrow specification. Both Aurora B and Polo are localized to the spindle midzone during metaphase and phosphorylate Pavarotti (Pav; MLKP1 in mammals, ZEN-4 in *C elegans*), a plus-end directed motor protein that is a component of centralspindlin. The other component of centralspindlin is the RacGAP Tumbleweed (Tum; MgcRacGAP50c in mammals, CYK-4 in *C elegans*). This complex is bound by the 14-3-3 proteins (ζ and ϵ in flies) which prevents the formation of stable microtubules (Douglas et al., 2010). Phosphorylation of 14-3-3 by Aurora B causes release of centralspindlin and this leads to clustering and stable accumulation of centralspindlin at the site of the spindle midzone/chromosomes. It is thought that this interaction is to prevent clustering at regions away from the spindle midzone as Aurora B only localizes at the spindle midzone.

Pav is a plus-end directed motor protein and during establishment of the cleavage furrow the centralspindlin complex travels along the microtubules to their ends. Once centralspindlin reaches the microtubule plus-ends, centralspindlin can interact with the cortex and establish the contractile ring at this position. Tum plays several roles once it is at the cortex. One role is to establish the connections

between the mitotic spindle, the contractile ring, and the cell cortex. At the plus ends of the microtubules, Anillin and Tum form a complex to link the mitotic spindle to the actomyosin contractile ring (D'Avino et al., 2008; Piekny and Glotzer, 2008; Tse et al., 2011). The complex of Anillin and Tum also interact with the plasma membrane by binding to phosphatidylinositol 4,5-bisphosphate (PIP_2) (Liu et al., 2012; Lekomtsev et al., 2012). The interaction between Tum and PIP_2 links the spindle to the cortex while the interaction between Anillin and PIP_2 link the contractile ring to the cortex. These interactions are important for the precise positioning of the cleavage furrow. For example, in Anillin mutants the cleavage furrow can actually migrate to a new position because it is not properly connected to the cell membrane (Piekny and Glotzer, 2008). Anillin has a second function to activate the septin, peanut which acts in the organization of the cytokinetic ring (Adam et al., 2000).

The RacGAP function of Tum is activated by phosphorylation by Polo kinase and once activated Tum can interact with and activate Pebble (Pbl; Ect2) (Wolfe et al., 2009). Pbl is a RhoGEF and once activated it causes Rho GTPase (Rho) to exchange GDP for GTP activating Rho. At this point, Rho activates several pathways: one leads to the localization and formation of myosin filaments at the cleavage furrow and the other establishes the actin cytoskeleton at the cleavage furrow. To activate myosin, Rho phosphorylates Rho kinase (Rok) which is then able to phosphorylate the regulatory light chain of myosin leading to filament formation. To establish the actin cytoskeleton, Rho activates the formin, Diaphanous (Dia), which leads to actin filament formation (Watanabe et al., 2008).

Once the contractile ring finishes contraction the cell must undergo abscission to sever the connection between the two daughter cells which may take up to

several hours (Schiel and Prekeris, 2010). At the site of the cleavage furrow the actomyosin contractile ring must be disassembled, the spindle midbody must be broken down, and the plasma membrane must be remodeled to separate the two cells.

Myosin recruitment, maintenance, and activity

Many of the proteins discussed above play a role in the completion of cytokinesis but they do not act to regulate myosin in the same ways. Proteins involved in the pathway regulating the cleavage furrow can act on myosin in one or more of the following capacities: recruitment of myosin to the equatorial region, maintenance of myosin at the equatorial region, or activation of myosin contractility at the equatorial region (Dean et al., 2005; Straight et al., 2005). The roles of the more important cleavage furrow components are summarized in Table 1.1.

TABLE 1.1. The Role of Cleavage Furrow Components in Myosin Recruitment, Maintenance, and Activity.

Furrow Component	Myosin Recruitment	Myosin Maintenance	Myosin Activity
Pav	−	+	+
Tum	−	+	+
Pbl	+	+	+
Anillin	−	+	+
Actin	−	+	+
Dia	−	−	+
Rho	+	+	+
Rho kinase	+	+	+

Bridge to Chapter II

In the preceding chapter I described the mechanisms that cells use to produce daughter cells of particular sizes: either equal-sized daughters or asymmetrically sized daughters. Typically cells used spindle-directed pathways but recently a spindle-independent mechanism has been described in *Drosophila* neuroblasts and *C. elegans* Q neuroblasts. In these systems, a basal myosin domain is required to produce asymmetric cytokinesis but the pathways that regulate this domain and the mechanism by which asymmetric cytokinesis is produced is not fully elucidated. In the following chapters, I describe the models that we tested to determine the mechanism by which the basal myosin domain produces asymmetric cytokinesis. One model is that the basal domain restricts growth at the basal pole causing that daughter cell to be smaller while the other is that the basal domain contracts on the mitotic spindle causing the cleavage furrow to reform at this point of contact. I also investigated that pathway that regulates the basal domain and if there are similarities to the pathway that regulates the cleavage furrow.

This dissertation includes previously published coauthored material.

CHAPTER II

ASYMMETRIC CORTICAL EXTENSION SHIFTS CLEAVAGE FURROW POSITION IN *DROSOPHILA* NEUROBLASTS

Reproduced with permission from Connell, M., Cabernard, C., Ricketson, D., Doe, C.Q., and Prehoda, K.E. (2011) Asymmetric cortical extension shifts cleavage furrow position in *Drosophila* neuroblasts. *Mol Biol Cell*, 22:4220-4226.

Author contributions: M.C., C.C., D.R., C.Q.D., and K.E.P. designed experiments; M.C. and C.C. performed experiments; M.C. and K.E.P. analyzed data; and M.C., C.C., C.Q.D., and K.E.P. wrote the paper.

Introduction

During development, asymmetric cell division is used repeatedly to generate daughter cells that differ in size and fate (Knoblich, 2008). Daughter cell size asymmetry, which may be important for maintaining progenitor growth potential (Joregensen and Tyers, 2004), can result from asymmetric positioning of the cleavage furrow (Glotzer, 2004). The site on the cortex where the cleavage furrow forms can be specified by the mitotic spindle (Oliferenko et al., 2009; von Dassow, 2009). For example, in the *Caenorhabditis elegans* zygote the spindle is displaced posteriorally at the end of metaphase and the furrow forms accordingly, leading to large anterior and small posterior daughter cells (Albertson, 1984; Keating and White, 1998; Glotzer, 2004). However, the position of the furrow depends not only on the site of furrow selection, but also on the relationship between the site of spindle specification and its position relative to the poles of the cell. Thus it

is possible that an asymmetric furrow could result from specification of a furrow site at the center of the cell followed by asymmetric movement of the cortex at the cell poles. The morphology changes during mitosis can be dramatic, prompting us to explore the role of polar cortical movements in furrow position. The study of furrow positioning has focused on how the site on the cortex that will become the cleavage furrow is selected by the mitotic spindle (Glotzer, 2004; von Dassow, 2009). The spindle pathway for furrow site selection is initiated at the central spindle by the centralspindlin complex consisting of the kinesin Pavarotti (ZEN-4 in *C. elegans*), the RacGAP Tumbleweed (CYK-4 in *C. elegans*), and the RhoGEF Pebble (ECT-2 in *C. elegans*). At the cell cortex, centralspindlin activates a narrow band of GTP-loaded Rho GTPase (Bement et al., 2006), ultimately leading to recruitment and activation of actomyosin to initiate cleavage furrow constriction. Astral microtubules can sharpen the site of furrow selection by inhibiting activation of Rho at the poles.

In addition to the spindle-directed equatorial constriction that occurs during cleavage furrowing, other morphological changes can happen late in mitosis. Symmetrically dividing cells, such as cultured S2 cells, round up at the beginning of mitosis but elongate at the poles late in anaphase (Hickson et al., 2006; Kunda et al., 2008; Rosenblatt, 2008) (Figure 2.1.A). The elongation that results from polar extension (i.e., outward displacement of the cortex) allows the spindle to expand into the polar regions as anaphase progresses (Rosenblatt et al., 2004; Hickson et al., 2006). The degree to which the polar cortex extends in cells that divide asymmetrically has been less characterized. Here, we use neuroblast asymmetric cell division as a model system for investigating the role of polar extension in cleavage furrow position and daughter cell size.

Drosophila neuroblasts are progenitors of the CNS, dividing to generate a larger apical cell that retains the neuroblast fate and a smaller basal ganglion mother cell (GMC) that assumes a differentiated fate (Doe, 2008; Knoblich, 2008). Neuroblasts divide rapidly, and daughter cell size asymmetry may be a mechanism for retaining sufficient resources to allow neuroblast self-renewal. The difference in fate of the two cells results from the polarization of fate determinants into separate apical and basal cortical domains that are precisely separated by the cleavage furrow (Knoblich, 2008). For example, the protein Miranda is localized to the basal cortex in metaphase and becomes segregated into the basal daughter cell as part of the machinery that confers GMC fate (Rolls et al., 2003; Lee et al., 2006; Atwood et al., 2007).

In addition to the spindle-directed pathway that controls targeting of furrow components such as centralspindlin to the equatorial cortex, neuroblasts possess a spindle-independent pathway that targets furrow components to the basal cortex (Cabernard et al., 2010). Shortly before the spindle directs recruitment to the equator, the spindle-independent pathway initiates contraction of the basal cortex (which becomes the GMC following abscission). A similar polar domain containing myosin II has recently been identified in *C. elegans* Q neuroblasts (Ou et al., 2010), which also divide to generate unequal-sized progeny, suggesting that the domain could be part of a common mechanism for daughter cell size asymmetry. Although the asymmetric contraction pathway is active in both *Drosophila* and *C. elegans*, and thus may be widely used in other systems, little is known about its mechanism. In particular, we know little about the cortical properties of the myosin-enriched (basal) and myosin-depleted (apical) neuroblast cortex.

Results

Neuroblasts elongate asymmetrically during anaphase

We imaged larval brain neuroblasts expressing a green fluorescent protein (GFP) fusion to the cortical marker Discs Large (Dlg-GFP) or myosin II regulatory light chain (Spaghetti squash in *Drosophila*; Sqh-GFP) as a cortical marker to measure polar extension during mitosis (where polar extension refers to displacement of the cortex at the poles, without regard to the underlying mechanism). In contrast to the equal polar extension observed in symmetrically dividing cells (Rosenblatt et al., 2004; Hickson et al., 2006), neuroblast cortical extension is highly asymmetric (Figure 2.1, B and C). The neuroblast apical cortex (associated with the larger daughter cell that retains the neuroblast fate) extends significantly during anaphase, whereas the basal cortex (associated with the smaller differentiated cell) undergoes very little extension (Figure 2.1, B and D). On average, the apical pole extends over threefold more than the basal pole (2.0 vs. 0.6 µm). Thus polar extension in neuroblasts in intact tissue is highly asymmetric.

To ensure that asymmetric cortical extension was not a consequence of physical constraints imposed by the surrounding tissue, we measured cortical extension in cultured embryonic neuroblasts. These cells are dissociated from their surrounding tissue but continue to undergo asymmetric divisions (Siegrist and Doe, 2006). We found that cultured embryonic neuroblasts underwent asymmetric polar extension similar to their counterparts in the larval brain (Figure 2.1.D). We conclude that neuroblast asymmetric extension is an intrinsic property of the asymmetric cell division. Thus anaphase cortical extension differs significantly between symmetrically dividing cells and asymmetrically dividing neuroblasts.

Whereas symmetrically dividing cells expand equally at both poles, neuroblast asymmetric divisions preferentially expand at the pole that becomes the larger daughter cell.

Asymmetric cortical extension is not caused solely by membrane synthesis

Neuroblast asymmetric cortical extension could occur by the preferential creation of new membrane at the apical surface. In this model the total surface area of the two daughter cells should be significantly larger than the surface area of the neuroblast before asymmetric cortical extension. To test this model, we measured the total surface area of the cell using three-dimensional reconstruction as a function of the cell cycle. We observed that the total surface area of the two daughter cells at the completion of cytokinesis is only ∼10% larger than the surface area of the metaphase neuroblast (Figure 2.1.E). In contrast, the difference in surface area of the two daughter cells is much more extreme (Figure 2.1.F). For example, the surface area of a representative neuroblast at metaphase was 923 μm^2, and upon completion of cytokinesis the resulting neuroblast and GMC had surface areas of 828 and 163 μm^2, respectively. Thus preferential membrane synthesis at the apical cortex is not sufficient to explain the asymmetric cortical extension that we observe, although it could contribute to the effect. Note that our measurements do not rule out a role for biased membrane flux.

Asymmetric cortical extension is independent of astral microtubules

To identify the cellular components responsible for cell-intrinsic asymmetric polar extension, we first focused on a possible role for the mitotic spindle, as it controls many of the morphological changes that occur during mitosis (von

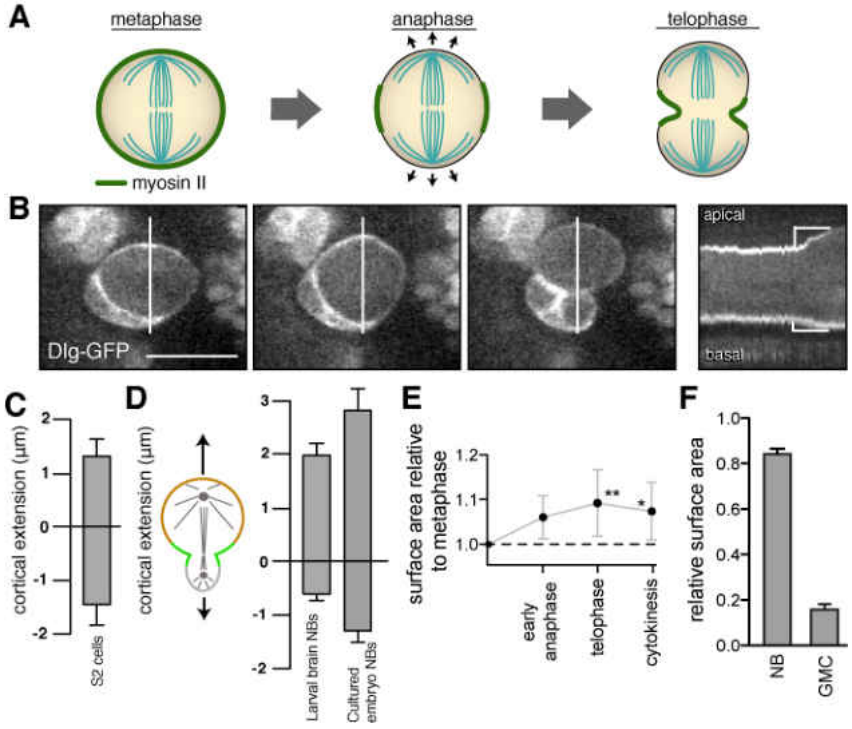

FIGURE 2.1. *Drosophila* neuroblasts undergo asymmetric polar elongation during anaphase. (A) Schematic of a symmetric division in which cortical extension is equal at both poles during anaphase. Myosin II is localized uniformly early in mitosis but becomes restricted to the equatorial region during anaphase. (B) Neuroblast cortical dynamics during mitosis using Dlg-GFP as a cortical marker. Selected frames from the movie are shown along with a kymograph of the entire division at 6-s intervals. The lines in the movie frames denote the section of the frame used for the kymograph. Cortical extension during anaphase is denoted by yellow brackets in the kymograph. The signal is enriched at the basal cortex because of contact with GMCs from previous divisions that also express GFP-Dlg (Supplemental Figure A.1). (C) Mean anaphase polar extension in cultured Drosophila S2 cells transiently expressing Cherry-Zeus. The edge of the cell was marked at the point at which cytoplasmic fluorescence was no longer observed. Error bars, 1 SD. (D) Quantification of anaphase cortical extension in wild-type neuroblasts. The mean cortical extension from metaphase to the end of anaphase is shown for the apical (top) and basal (bottom) cortexes (NB, neuroblast). (E) The surface area of dividing neuroblasts measured using three-dimensional reconstruction normalized to that at the end of metaphase. The time points for measurements were early anaphase (completion of cortical extension), telophase (initiation of furrowing), and cytokinesis (completion of furrowing). (F) Mean relative surface areas of the daughter neuroblast (NB) and GMC that results from a neuroblast asymmetric cell division measured as in E at the completion of furrowing.

Dassow, 2009). At the poles, astral microtubules contact the cortex and could be responsible for controlling the asymmetric polar extension observed in neuroblasts. For example, asymmetric growth of the apical spindle could push the apical cortex outward. We examined whether astral mictrotubules are required for the difference in polar extension seen at the apical and basal neuroblast cortex by examining sas^4 mutants, which lack astral microtubules (Basto et al., 2006). As shown in (Figure 2.2.A), the cortical extension in sas^4 occurs predominantly at the apical pole, similar to wild-type neuroblasts. We conclude that astral microtubules are not required for asymmetric cortical extension.

Cortical extension occurs at the onset of apical myosin II depletion

We examined the localization of the cortical factor myosin II to determine whether it could be important for polar extension in neuroblasts. In symmetrically dividing cells, myosin II is uniformly cortical in prophase but becomes confined to the equatorial region by late anaphase (Rosenblatt et al., 2004). Neuroblasts exhibit a similar pattern of myosin II localization, except that myosin II is retained at the basal cortex during anaphase along with Anillin and Pav, which are normally restricted to the furrowing region (Cabernard et al., 2010). In examining the localization of myosin II, we noticed a striking correlation with the loss of Sqh-GFP signal at the apical cortex and the onset of cortical extension, whereas myosin II remained at the basal cortex where extension was limited (Figure 2.2, B–D). Preferential apical clearing is not observed for the control proteins Dlg-GFP and Moe-RFP (Supplemental Figure A.2). Furthermore, asymmetric cortical extension is not dependent on Sqh overexpression, as it also occurs in sqh mutants expressing Sqh-GFP (Figure 2.2.C). The retention of myosin II on the basal cortex, along with

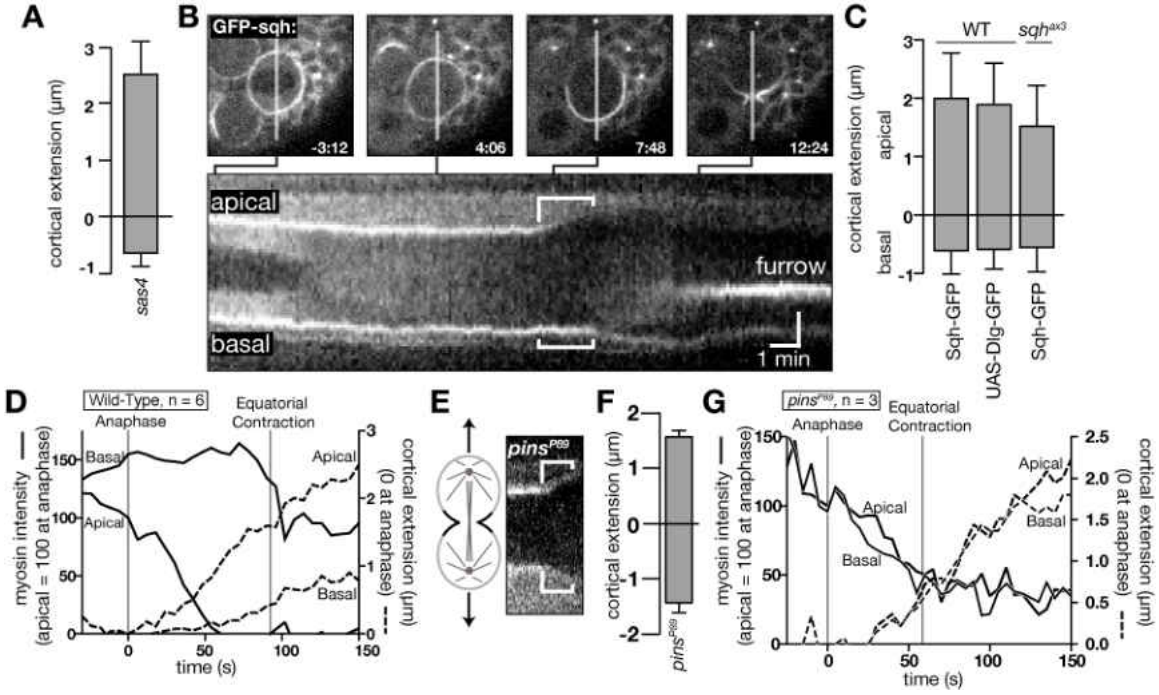

FIGURE 2.2. Basal myosin II is required for asymmetric polar elongation. (A) Mean anaphase polar extension in *sas*^{4} mutant neuroblasts. Error bars, 1 SD. (B) Kymograph of myosin II (Sqh-GFP) in a wild-type larval neuroblast imaged at 6 s intervals. Top, selected frames with time relative to nuclear envelope breakdown. A line marks the section of the frame used for the kymograph. Anaphase cortical extension is denoted by brackets in the kymograph. (C) Quantification of apical and basal cortical extension in *sqh*^{ax3}; sqh-GFP (larval neuroblasts), sqh-GFP, and *worniu*-Gal4; UAS-Dlg-GFP neuroblasts. Error bars, 1 SD. Anaphase onset was determined using spindle (Jupiter-cherry) or chromosome (His2A-mRFP) markers. (D) Time dependence of cortical myosin signal and cortical position for wild-type neuroblasts. Dashed lines indicate the cortical position at each pole relative to the position at anaphase start. Solid lines indicate the intensity at each pole relative to the apical cortical intensity at anaphase start (determined as in C). Equatorial contraction indicates the time point at which the initiation of furrow ingression was observed. (E) Kymograph of Sqh-GFP in *pins*^{P89} mutant neuroblasts. Brackets denote polar extension during anaphase. (F) Quantification of anaphase cortical extension in pins mutant neuroblasts. Error bars, 1 SD. (G) Time dependence of cortical myosin signal and cortical position for *pins*^{P89} mutant neuroblasts. Annotations as in D.

the limited cortical extension at this location, prompted us to hypothesize that the basal domain containing furrow components inhibits cortical extension, limiting cortical extension to the apical cortex.

Asymmetric cortical extension requires a G-protein/Partner of Inscuteable regulated basal furrow domain

As the onset of cortical extension is highly correlated with the loss of myosin II, we hypothesized that the basal furrow domain containing myosin II, Anillin, and Pavarotti, might be responsible for the preferential extension of the apical cortex. This domain is not regulated by the spindle but is instead controlled by cortical polarity factors such as Partner of Inscuteable (Pins; Cabernard et al., 2010). We examined cortical extension in symmetrically dividing pins mutants to determine how loss of the basal myosin domain influences extension. We confirmed that neuroblasts lacking Pins fail to form the basal myosin domain (Cabernard et al., 2010; Figure 2.2.E). We find that these neuroblasts extend equally at both the apical and basal poles during anaphase (Figure 2.2.F and G). We conclude that Pins, which is required for the basal furrow domain, is also required for asymmetric polar extension. The known role of Pins in regulating asymmetric daughter cell size (Yu et al., 2000) suggests that these three processes – the basal furrow domain, asymmetric polar elongation, and daughter cell size asymmetry – are tightly coupled.

To further test the relationship between the basal furrow domain, asymmetric cortical elongation, and daughter cell size, we examined another genetic background in which neuroblasts divide symmetrically. Overexpression of the Pins-binding Gαi protein induces a high rate of symmetric divisions (Yu et al., 2000; Schaefer et al., 2001; Nipper et al., 2007). We imaged larval brain neuroblasts expressing Gαi under the control of the neuroblast-specific *worniu*-Gal4 driver to determine whether elevated Gαi levels concurrently alter the basal furrow domain and cortical extension prior to symmetric cell division. We found that the basal furrow domain

failed to form in approximately half the neuroblasts, and neuroblasts lacking the domain expanded equally at both poles during anaphase (Figure 2.3.A–C and E). To determine whether the effect on the basal furrow domain and cortical extension is due to a greater pool of activated Gαi, we examined cortical extension in neuroblasts expressing the activated Q205L Gαi mutant (UAS-GαiQ205L driven with *worniu*-Gal4), which does not bind Pins or the G$\beta\gamma$ subunits (Schaefer et al., 2001). These neuroblasts divided normally, suggesting that the ability to bind Pins is required for Gαi-induced symmetric cell division (Figure 2.3.C and D). Thus heterotrimeric G-proteins and Pins are required for the basal furrow domain and asymmetric polar extension and daughter cell size.

Asymmetric cortical extension requires alignment of the spindle with the basal furrow domain

We examined cortical extension in *mud* mutants to examine the consequence of decoupling the spindle-directed and basal furrows on polar extension. Mud orients the spindle with the polarity axis, and loss of mud function leads to randomization of spindle position (Guan et al., 2000; Bowman et al., 2006; Izumi et al., 2006; Siller et al., 2006). In *mud* mutant neuroblasts with misaligned spindles, structures resembling polar bodies form at the basal furrow domain prior to equatorial contraction from the spindle-directed furrow (Cabernard et al., 2010). We examined cortical extension in this context to determine whether the basal furrow domain must be aligned with the spindle for asymmetric cortical extension. We found that although the cortex opposite the basal furrow domain begins to expand, subsequent spindle-induced equatorial furrowing overcomes this extension and ultimately leads to symmetric extension at the spindle poles and daughter

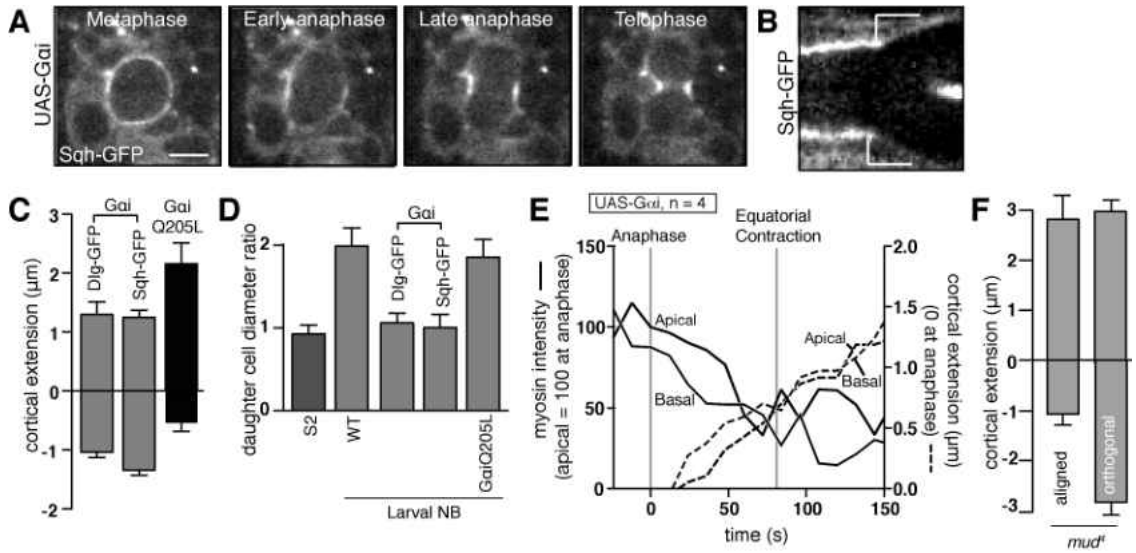

FIGURE 2.3. G-protein signaling regulates the basal furrow domain. (A) Myosin II (Sqh-GFP) localization in larval brain neuroblasts expressing Gαi using *worniu-Gal4*; UAS-Gαi. Images shown were taken 12 s apart. Scale bar, 5 µm. (B) Kymograph of Sqh-GFP signal across the poles from movie in A. Cortical extension is marked by the white lines. (C) Mean polar elongation for neuroblasts expressing Gαi or Gαi Q205L, a constitutively active variant that does not bind G$\beta\gamma$ or Pins. Cortical extension for Gαi is shown with two different cortical markers (Sqh-GFP or Dlg-GFP). Error bars, 1 SD. (D) Comparison of daughter cell size ratio for various cell types examined here. For asymmetrically dividing cells, this ratio was determined by dividing the diameter of the apical cell by the diameter of the basal cell. (E) Time dependence of cortical myosin signal and cortical position for neuroblasts expressing Gαi. Dashed lines indicate the cortical extension at each pole, whereas solid lines indicate the normalized intensity at each pole (as in Figure 2.2.D). (F) Anaphase cortical extension for *mud*^{4} mutants where the spindle was aligned with basal myosin domain or orthogonal to it.

cell size (Figure 2.3.F). Thus coupling of both spindle-independent and spindle-dependent contractile pathways is required for asymmetric polar extension.

Asymmetric cortical extension does not require spindle-directed equatorial contraction

Our results suggest that the basal furrow domain is required for asymmetric cortical extension. The basal domain could bias cortical extension toward the apical pole in at least two possible ways. The basal domain could increase the

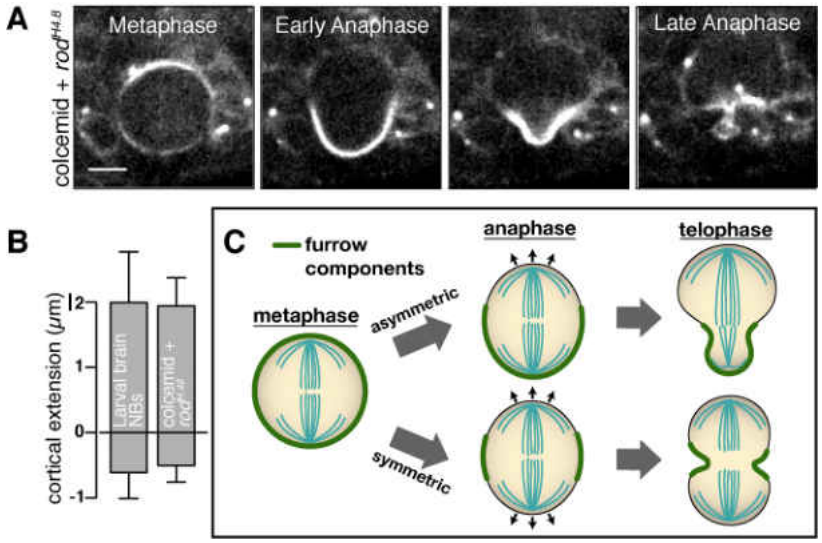

FIGURE 2.4. Asymmetric cortical extension does not require spindle-induced equatorial contraction. (A) Sequence of a Colcemid treated *rod*$^{-}$ mutant neuroblast expressing Jupiter-cherry to ensure the absence of a spindle. Scale bar, 10 µm. (B) Quantification of cortical extension in Colcemid-treated rod mutants. Error bars, 1 SD. (C) Model for the role of the basal furrow domain in daughter cell size asymmetry.

rigidity of the basal cortex, thereby focusing cortical extension induced by spindle-induced equatorial contraction to the apical cortex. Alternatively, the basal domain itself could be responsible for extension at the apical cortex. To distinguish between these models, we treated neuroblasts with Colcemid to depolymerize microtubules, thereby inhibiting formation of the spindle and the spindle-directed furrow (Brinkley et al., 1967; Cabernard et al., 2010). To allow these neuroblasts treated with Colcemid to pass through the spindle checkpoint without a spindle, the Colcemid treatments were done in a *rod*$^{-}$ background (Basto et al., 2000; Chan et al., 2000; Savoian et al., 2000; Cabernard et al., 2010). The presence of the basal furrow domain alone is sufficient for asymmetric cortical extension, as anaphase cortical extension is indistinguishable from wild-type neuroblasts (Figure 2.4.A and B). Thus we conclude that spindle-directed equatorial contraction is not required to produce asymmetric cortical extension.

Discussion

Morphological changes that occur during mitosis involve both positive and negative signals that emanate from the mitotic spindle. For example, in *C. elegans* the first division yields unequal-sized daughter cells (Albertson, 1984; Keating and White, 1998; Glotzer, 2004). At the metaphase-to-anaphase transition the spindle midzone is displaced toward the posterior end of the zygote and is believed to positively influence furrow formations, whereas astral microtubules repress furrowing at the poles (Albertson, 1984; Keating and White, 1998; Glotzer, 2004). Thus asymmetric positioning of the spindle before anaphase ultimately leads to a displaced furrow and unequal-sized daughter cells. In neuroblasts, however, the spindle is symmetrically positioned before anaphase, suggesting that other mechanisms are responsible for neuroblast asymmetric division (Cai et al., 2003; Kaltschmidt et al., 2000; Siller et al., 2006). Recently, components normally restricted to the cleavage furrow were found to be localized to the neuroblast basal cortex (Cabernard et al., 2010). We found that this basal furrow domain restricts cortical extension that normally happens at both poles during the division of many small, symmetrically dividing cells. This restriction of cortical extension limits the size of the future basal cell while allowing the future apical cell to expand during anaphase (Figure 2.4.C).

The contribution of asymmetric cortical extension to daughter cell size explains why *asl* and *cnn* mutants, which lack astral microtubules, divide asymmetrically although they have a symmetric spindle (Bonaccorsi et al., 1998; Basto et al., 2006). We argue that the asymmetric spindle poles (large apical pole, small basal pole) observed in wild-type neuroblasts late in the cell cycle (Fuse et al., 2003) are a consequence, not a cause, of asymmetric cortical extension. Lack

of basal cortical extension prevents the spindle from growing at this pole, whereas it is free to grow at the apical pole. In fact, in S2 cells where cortical extension has been inhibited at both poles by RNA interference knockdown of Rho kinase (Hickson et al., 2006), spindles become bent and distorted during anaphase. We propose that asymmetric cortical extension in neuroblasts channels spindle growth into the apical pole, resulting in a highly symmetric spindle. The basal myosin domain does not passively halt the extension at the basal pole by acting against the forces produced by spindle-directed equatorial contraction, but instead is directly responsible for asymmetric cortical extension. Nevertheless, the basal and spindle-directed myosin domains must work together for asymmetric cortical extension to occur (Figure 2.2.F).

How is the basal furrow domain regulated? Several components that function upstream of myosin II in the canonical furrow (e.g., Pav and Anillin) are also present in the basal furrow domain (Cabernard et al., 2010). Previous work showed that the polarity protein Pins is required to establish a basal myosin domain, whereas Gαi mutants do not perturb the domain (Cabernard et al., 2010). A linear model in which G$\beta\gamma$ promotes the basal myosin domain but is inhibited by Gαi, which is in turn inhibited by Pins, is consistent with these observations, and our data provide further support. Pins binds Gαi such that it is dissociated from G$\beta\gamma$, so in cells that lack Pins, heterotrimeric G-protein complex assembly is favored and both Gα and G$\beta\gamma$ activity are reduced. As Gαi mutants have normal basal furrow domains (Cabernard et al., 2010), Gαi is not required for the basal furrow domain, suggesting that G$\beta\gamma$ is the relevant furrow domain regulator. The Gαi overexpression results presented here are also consistent with this model. We observed that expression of Gαi, which would inactivate any free G$\beta\gamma$, leads to

loss of the basal furrow domain. However, expression of constitutively active Gαi, which does not bind G$\beta\gamma$ but does bind downstream effectors, has no effect on the furrow domain. Thus we propose that G$\beta\gamma$ activity is essential for establishing the basal myosin domain, asymmetric cortical extension, and unequal daughter cell size. Future work will be directed toward rigorously testing this model and identifying the link between polarity proteins and myosin.

Materials & Methods

Fly lines

All mutant chromosomes were balanced over CyO actin:GFP, TM3 actin:GFP, Ser, e, or TM6B Tb. We used Oregon R as wild type and the following mutant chromosomes and fly strains: *Sqh:GFP* (Royou et al., 2002), *worGal4* (Albertson and Doe, 2003), *UAS-Cherry:Jupiter* Cabernard and Doe (2009), *worGal4* and *UAS-Dlg:eGFP* (Koh et al., 1999), *UAS-Gαi* and *UAS-GαiQ205L* (Schaefer et al., 2001; kindly provided by J.A. Knoblich), mud^4 (Guan et al., 2000), $pins^{P89}$ (Yu et al., 2000), *FRT82B Sas-4*^{M} (Basto et al., 2006), $rod^{H4.8}$ (Basto et al., 2000), *UAS-His2A-mRFP* (Emery et al., 2005), *UAS-moe-RFP* (Schwave et al., 2005), and *UAS-Jupiter-GFP* (Karpova et al., 2006). All crosses were performed at 25°C except *UAS-Gαi* crosses, which were performed at room temperature and transferred to 30°C approximately 8 h prior to imaging.

Embyronic neuroblast culture

Primary cell cultures were made from embryos aged 6–8 h as previously described (Grosskortenhaus et al., 2005). They were then prepared for live imaging

by resuspension in Chan and Gehrings balanced saline solution supplemented with 2% FBS.

Live imaging

Second or third larval brains were prepared for imaging as previously described (Siller et al., 2005). Five to nine Z steps were collected at 1– to 2–µm intervals every 6–12 s. Live imaging was performed using a spinning disk confocal microscope equipped with a Hamamatsu EM-CCD camera (Hamamatsu, Japan) using a 63 x 1.4 numerical aperture oil immersion objective. Pixel intensity measurements were performed using ImageJ (National Institutes of Health, Bethesda, MD). A linear photobleaching correction was added, using the intensity of a region outside the cell as a reference. Cortical extension was determined by measuring the position of the cell edges at the poles with a section from the middle of the cell as determined by examination of sections throughout the cell.

Colcemid treatment was performed on the strain *+; worGal4, UAS-Cherry:Jupiter, Sqh:GFP; rod*$^{H4.8}$ (Cabernard et al., 2010), using a final concentration of 0.1 µM/mL, with live imaging beginning immediately after treatment.

Three-dimensional reconstructions for surface area analysis were done using the BoneJ plugin for ImageJ with a sampling value of 2 (Doube et al., 2010).

Cell culture

Drosophila S2 cells were maintained at room temperature in Schneiders medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (Sigma-Aldrich). Cells were transiently transfected with pMT-Zeus-

Cherry with Effectene (Qiagen, Valencia, CA) according to the manufacturers instructions, and expression was induced with 0.5 M copper sulfate 20 h prior to imaging. Immediately before imaging, cells were resuspended in Schneiders medium supplemented with 10% fetal bovine serum.

Bridge to Chapter III

Chapter II demonstrated the discovery of a basal myosin domain that is required for asymmetric cytokinesis in *Drosophila* neuroblasts. We proposed a model that the basal domain leads to asymmetric cytokinesis by restricting the cortical extension at the basal pole. This allows the apical daughter cell to extend freely leading to its larger size. Based on more recent observations, Chapter III discusses a new model by which the basal domain could lead to asymmetric cytokinesis. The model discussed therein suggests that the contractile function is not to hinder extension but to bring the cortex in contact with the spindle at a position that is different from the equatorially specified furrow.

CHAPTER III

THE CLEAVAGE FURROW IS REPOSITIONED DUE TO CONTRACTION OF THE BASAL MYOSIN DOMAIN ONTO THE MITOTIC SPINDLE

Introduction

Cells divide to produce daughter cells of different sizes through a process of asymmetric cytokinesis. This process is important in development when stem cells are dividing to produce the different types of daughter cells needed to create all the different tissues of the organism (Knoblich, 2008). While the exact reason for this process is not established, it is hypothesized that the stem cell retains the larger size so that it can divide again in a shorter timeframe because it doesn't have to spend time in the growth portion of the cell cycle (Joregensen and Tyers, 2004).

In many cell types, asymmetric cytokinesis is produce by localizing the spindle closer than one pole rather than in the middle of the cell. This changes the position of the furrow as the mitotic spindle provides the signals that produce the cleavage furrow (Glotzer, 2004). Recently a new method of creating asymmetric cytokinesis in cells has been discovered in *Drosophila* neuroblasts and *C. elegans* Q neuroblasts (Cabernard et al., 2010; Ou et al., 2010). These cells have a spindle-independent mechanism that relies on the presence of a myosin domain at one pole of the cell. In mutants that lose the basal myosin domain such as *pins* mutants in *Drosophila* and pig-1 in C elegans, the cells divide symmetrically to produce daughter cells of equal sizes.

We have previously proposed a model by which the basal myosin domain leads to asymmetric cytokinesis in *Drosophila* neuroblasts. In this model the basal

domain restricts cortical extension at the basal pole during anaphase and prior to equatorial contraction (Connell et al., 2011). This would effectively localize the spindle to a position that is biased towards one pole of the cell. We confirmed this model by over-expression of Gαi which leads to symmetrically dividing neuroblasts. In these mutants there is equal cortical extension at both poles and the daughter cells are of equal sizes. It was also proposed that the *C. elegans* Q neuroblasts also produce asymmetric cytokinesis by the contractile forces of the myosin domain restricting the size of the daughter cell that contains it (Ou et al., 2010). A second possibility was that the difference in apical daughter cell size is due to an unequal addition of membrane to the apical pole during mitosis. We have shown that there is no significant gain of cell membrane during this period to account for the difference in daughter cell size ruling out this mechanism (Connell et al., 2011).

Another possible model by which the basal myosin domain could cause asymmetric cell division in *Drosophila* neuroblasts is through a mechanism by which the contraction of the basal domain causes it to interact with the mitotic spindle at a position other than the spindle midzone. This interaction with the spindle would cause the mitotic spindle to specify the furrow in this location (Oliferenko et al., 2009; von Dassow, 2009). It has previously been shown that if the position of the mitotic spindle is changed during mitosis a Rho activity zone will form at the midzone of the spindle and induce a furrow after it was repositioned (Bement et al., 2006). The furrow that had originally be established does not complete and disperses due to the need of positive feedback from the spindle. If a region of non-equatorial cortex is moved so that it comes in contact with the mitotic spindle a Rho activity zone forms there and myosin is recruited.

In this chapter, we investigated the model wherein the basal domain contacting the spindle at a position basal to the spindle midzone leads to a shift in the localization of proteins that would normally localize to the spindle midzone and thereby causing the furrow to be specified at a region biased towards the basal pole.

Results

14-3-3 proteins are required for apical depletion of myosin

14-3-3 proteins(ζ and ϵ in flies) are required for proper formation of the cleavage furrow and are known to regulate the proper bundling of the spindle microtubules through regulation of Pavarotti (Douglas et al., 2010). Additionally, in *Dictyostelium* it has been shown that 14-3-3 proteins interact with myosin directly and in 14-3-3 mutants the myosin forms more punctate structures (Zhou et al., 2010), so we used RNAi to determine if they are required for the basal localization of myosin in *Drosophila* neuroblasts. Visualizing myosin localization (Sqh-GFP) in 14-3-3ζ and ϵ mutants, we found that myosin localized to the basal domain similarly to wild-type neuroblasts, but myosin was retained at the apical pole in both ϵ and ζ mutants (Figure 3.1.A and B). The average time that myosin remains at the apical cortex after anaphase onset in wild-type neuroblasts is 47.60 $\pm$ 16.58 s (Figure 3.2.A). For 14-3-3 ζ and ϵ mutants the average time was 78.40 $\pm$ 33.29 s and 111.4 $\pm$ 100.3 s respectively. In addition to the myosin phenotype several other phenotypes were observed: bent spindles, multipolar spindles, and misaligned spindles in respect to the basal myosin domain. Not all neuroblasts exhibited defects in myosin depletion, potentially due to poor penetrance of the RNAi as when we attempted to increase expression the RNAi become lethal. From

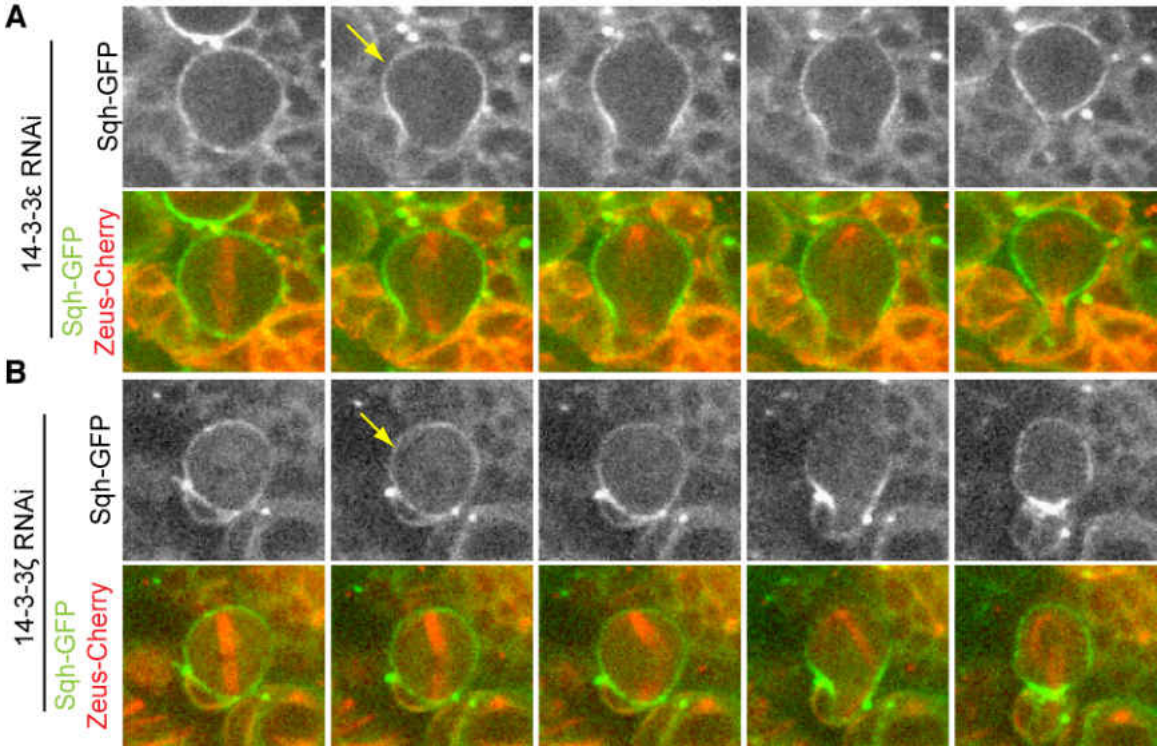

FIGURE 3.1. 14-3-3 proteins are required for loss of apical myosin. (A) Myosin localization in 14-3-3ϵ RNAi neuroblasts. RNAi constructs were driven using *worniu*-Gal4. Yellow arrows indicate retention of apical myosin. Images were taken every 12 s. (B) Myosin localization in 14-3-3ζ RNAi neuroblasts.

this data we can conclude that 14-3-3 proteins are involved in the apical depletion of myosin at anaphase onset in *Drosophila* neuroblasts.

Asymmetric cortical extension is not required for asymmetric cell division

Our previous model posited that asymmetric cytokinesis in *Drosophila* neuroblasts was caused by asymmetric cortical extension during anaphase prior to equatorial contraction. Wild-type neuroblasts extended at the apical pole 2-3 times more than at the basal pole, while neuroblasts over-expressing Gαi that lost basal myosin had equal extension at both poles (Connell et al., 2011). Based on that model, 14-3-3 mutants that retain apical myosin should also divide symmetrically

due to the restriction of cortical extension at the apical pole restricting the daughter cell size to a similar extent as the basal daughter cell. We determined the daughter cell ratio by taking the area of the largest cross section rather than the typical measurement of diameter used in neuroblasts because the 14-3-3 mutants tended to be distorted. We found that the 14-3-3 mutant neuroblasts divided asymmetrically and the daughter cell size ratio was not significantly different from that seen in wild-type (Figure 3.2.B).

From visual observation the spindle appears to crash into the apical pole suggesting restriction of cortical extension but perhaps the reason that the cells divide symmetrically is that the myosin is not contractile and does not restrict extension. We measured cortical extension at each pole and we found that 14-3-3 mutants had equal cortical extension at each pole prior to equatorial contraction while wild-type neuroblasts experienced ∼2-3 times more extension at the apical pole than at the basal pole (Figure 3.2.C). This demonstrates that apical myosin is able to to restrict extension until it eventually depletes and the cortex is able to extend. By the end of cytokinesis, the total extension at both poles is equal to that seen in wild-type(Figure 3.2.C). This data demonstrates that neuroblasts can divide symmetrically when cortical extension is hindered at the apical pole and both poles extend equally during anaphase prior to equatorial contraction. While the cortex does reach full wild-type extension, the asymmetric extension occurs after equatorial contraction begins and the furrow is positioned.

The cleavage furrow does not form at the spindle midzone

As asymmetric cortical extension is not required for asymmetric cytokinesis in *Drosophila* neuroblasts and previous research had suggested that there was

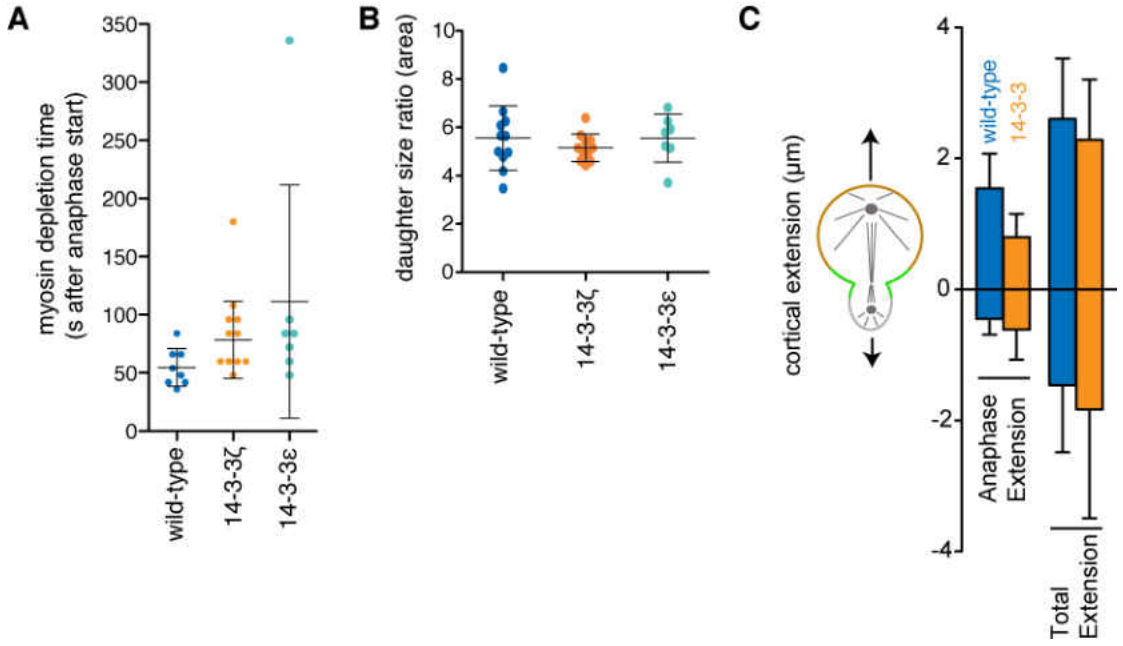

FIGURE 3.2. 14-3-3 mutant neuroblasts produce asymmetric divisions but demonstrate equal anaphase cortical extension (A) Myosin depletion after anaphase onset is delayed in 14-3-3 mutants. Time point zero represents the start of anaphase as determined using Zeus-cherry. Error Bars, 1 SD. (B) Daughter size ratio in wild-type and 14-3-3 mutant neuroblasts. Ratio was determined by determining the area of each daughter cell for the largest cross section. (C) Cortical extension in 14-3-3 mutant neuroblasts that retain apical myosin. Anaphase extension is the amount of extension that occurred prior to equatorial contraction and total extension is the total amount of cortical extension that has occurred at the end of cytokinesis

asymmetry in the mitotic spindle (Fuse et al., 2003), we looked at the movement of the mitotic spindle to determine if there was asymmetry in the spindle to account for the asymmetric cytokinesis. Tracking the position of the centrosomes in relation to the metaphase plate, we found that each centrosome moved an equal distance from the metaphase plate prior to equatorial contraction, making the spindle midzone and the point halfway between the two centrosomes still at the position of the metaphase plate (Figure 3.3.A). This suggests that the furrow should still form at the location of the metaphase plate as previous work in *C. elegans* has demonstrated that the furrow receives signals from the spindle midzone and the

asters to form at a position halfway between the two centrosomes (Bringmann and Hyman, 2005).

In neuroblasts we found that the cleavage furrow actually forms several microns away from the metaphase plate at a position biased towards the basal pole(Figure 3.3.B). We also determined the location of the cleavage furrow relative to the metaphase plate in 14-3-3 mutants to see if this was affected by the retention of apical myosin. We found that the furrow forms at approximately the same distance from the metaphase plate in 14-3-3 mutants as in wild-type (-2.126 ± 0.991 µm for 14-3-3 mutants vs -1.833 ± 1.029 µm for wild-type neuroblasts) (Figure 3.3.B). As the extension is restricted prior to equatorial contraction, the cleavage furrow position does not appear to rely on the amount of cortical extension. Gβ RNAi neuroblasts divide symmetrically and the average furrow position is -0.425 ± 0.485 µm (Figure 3.3.B) away from the position of the metaphase plate indicating this shift in furrow position requires the presence of the basal myosin domain.

Spindle microtubules contact the cortex at the point of basal domain contraction rather than at the location of the metaphase plate

Spindle microtubules specify the location of the cleavage furrow through interactions between tumbleweed (the mitotic spindle), anillin (the contractile ring) and PIP_2 (the plasma membrane) (D'Avino et al., 2008; Piekny and Glotzer, 2008; Liu et al., 2012; Lekomtsev et al., 2012). We imaged microtubules using zeus-GFP to determine where they interact with the cortex during cytokinesis in *Drosophila* neuroblasts (Figure 3.3.C). At metaphase the spindle appeared symmetric with few contacts with the cortex at either pole. As the basal domain contracts the spindle

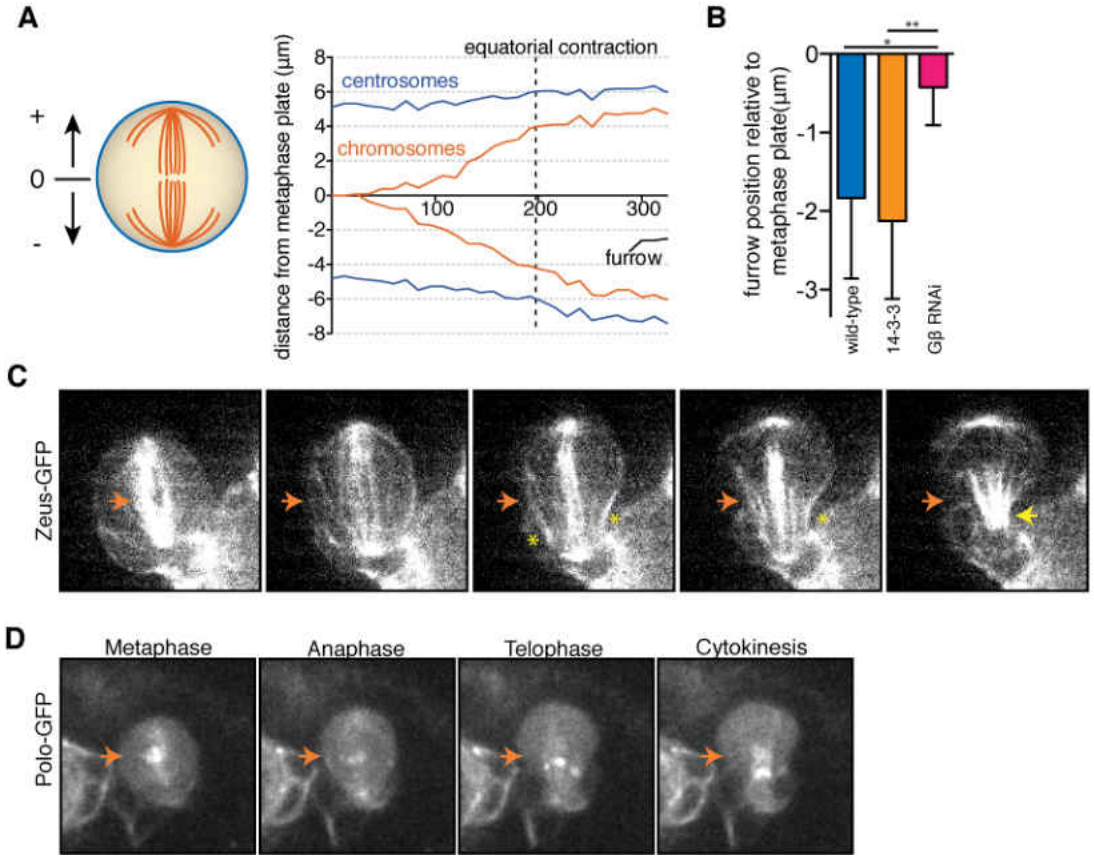

FIGURE 3.3. The cleavage furrow does not form at the spindle midzone. (A) Distance the centrosomes and chromosomes travel from the metaphase plate during mitosis. Time zero represents the frame immediately before anaphase onset which is when the chromosomes begin to pull apart. Positive values are positions apical to the metaphase plate and negative values are distances basal to the metaphase plate. n = 5. (B) Distance of the furrow from the position of the metaphase plate in wild-type, 14-3-3 mutant and Gβ13F mutant larval neuroblasts. Error bars represent 1 S.D. (C) Microtubule localization in wild-type neuroblasts. Zeus-GFP was used to mark the microtubules. Orange arrow indicates the position of the metaphase plate and yellow arrow indicates the position of the cleavage furrow. Yellow stars highlight regions of microtubule interactions with cortex. (D) Polo (Polo-GFP) localization in larval neuroblasts. Orange arrow indicates the location of the metaphase plate. Images were taken 12 s apart. Scale bar, 10 µm

microtubules begin to contact the cortex more at the basal pole than at the apical pole, specifically at the region of contraction at the basal domain. The cleavage furrow eventually forms at the region where microtubule contacts were made.

Localization of spindle midzone proteins shifts towards the basal pole as the basal myosin domain contracts

As spindle microtubules contact the cortex away from the spindle midzone, we determined if the localization of components that localize to the spindle midzone and establish the cleavage furrow is affected by the basal myosin domain. Polo kinase is a good candidate as it localizes to the spindle midzone where it interacts with with centralspindlin (Wolfe et al., 2009) among others. Using Polo-GFP, Polo was found to localize to the chromosomes at metaphase and in early anaphase it travels with the chromosomes towards the centrosome (Figure 3.3.D). As anaphase progresses, Polo reappears at the position of the metaphase plate but as the basal domain contracts, Polo localization shifts more basally eventually colocalizing with the furrow. This shift in Polo localization is not seen in symmetrically dividing cells (Moutinho-Santos et al., 1999). Previously published work has shown that Pav-GFP also has a similar localization pattern (Cabernard et al., 2010) and work discussed in Chapter IV demonstrates this migration does not occur in symmetrically dividing mutants. This supports the hypothesis that the basal domain is required for the shift in localization of the furrow components.

The basal myosin domain is composed of contiguous domains that act independently

As the equatorial domain established by the mitotic spindle and the basal myosin domain appear contiguous or even overlap at the cell cortex. We set out to determine if these regions have similar dynamics as previous work has shown that the the basal domain contracts even when the equatorial population of myosin has been removed through colcemid-induced disassembly of the mitotic spindle (Cabernard et al., 2010; Connell et al., 2011).

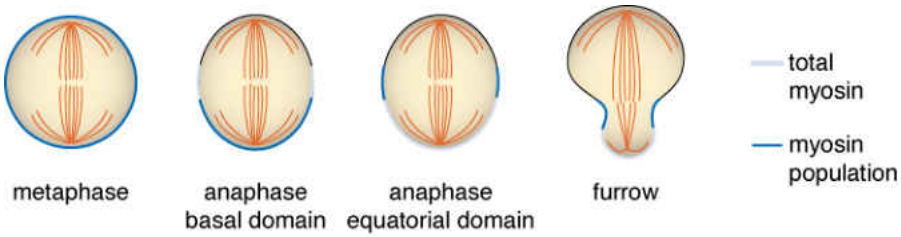

FIGURE 3.4. Multiple populations of myosin exist during mitosis. Schematic representing the different regions of myosin that exist during mitosis.

If the basal myosin domain is composed of two separate populations of myosin the more basal population would contract at a position that is more basal to the center of the cell. The edge of the basal domain would then restrict the size of the daughter cells.

Previous work in symmetrically dividing cells has investigated the dynamics of myosin during cytokinesis and found that myosin populations at the cleavage furrow became more stable as cytokinesis propressed (Uehara et al., 2010). This was indicated by the rate of recovery in photobleaching experiments. As cytokinesis progressed the recovery after photobleaching decreased. they were unable to recover from photobleaching (Uehara et al., 2010). To look at the dynamics of the basal domain vs. the equatorial domain we used photobleaching to determine the recovery rates. We bleached multiple regions in the neuroblasts as depicted in Figure 3.4.

We first photobleached the metaphase populations and cleavage furrow population in sqh^{ax3}; *sqh-GFP* neuroblasts. Sqh^{ax3} is a null allele and is rescued by sqh-GFP so the only myosin present is tagged with GFP. It has been shown previously that metaphase myosin recovers almost completely and at a much higher rate than the myosin found at the cleavage furrow (Uehara et al., 2010). Our results supported this data with the metaphase population recovering almost

completely with a mobile fraction of 91.07% and a $t_{1/2}$ of 20.44 ± 4.11 s (Figure 3.5.A,C). On the other hand, the cleavage furrow population only had a mobile fraction of 54.34% and a $t_{1/2}$ of 54.94 ± 11.02 s.

During anaphase we bleached both the basal region and the equatorial region. The basal region behaved similarly to the cleavage furrow with a mobile fraction of 51.32% and a $t_{1/2}$ of 29.17 ± 11.01 s while the equatorial region behaved similarly to the metaphase population of myosin with a mobile fraction of 123.16% and a $t_{1/2}$ of 81.43 ± 19.86 s (Figure 3.5.B,C).

These data suggests that the myosin domain seen during anaphase is composed of two populations: the equatorial region and the basal region, and both populations have different dynamics. As the contractile elements do not extend to the center of the cell, it suggests that daughter cell size could be dictated by the size of the basal myosin domain.

The higher rate of turnover at the equatorial region suggests that this region is not as contractile. This idea that the higher rate of turnover correlates to less contractility is supported by data that shows that furrowing cells with enhanced myosin II turnover exhibit slower furrowing in dividing HeLa cells (Kondo et al., 2011). The slower furrowing is due to the expression of a non-phosphorylatable regulatory light chain which would reduce the contractile activity of myosin.

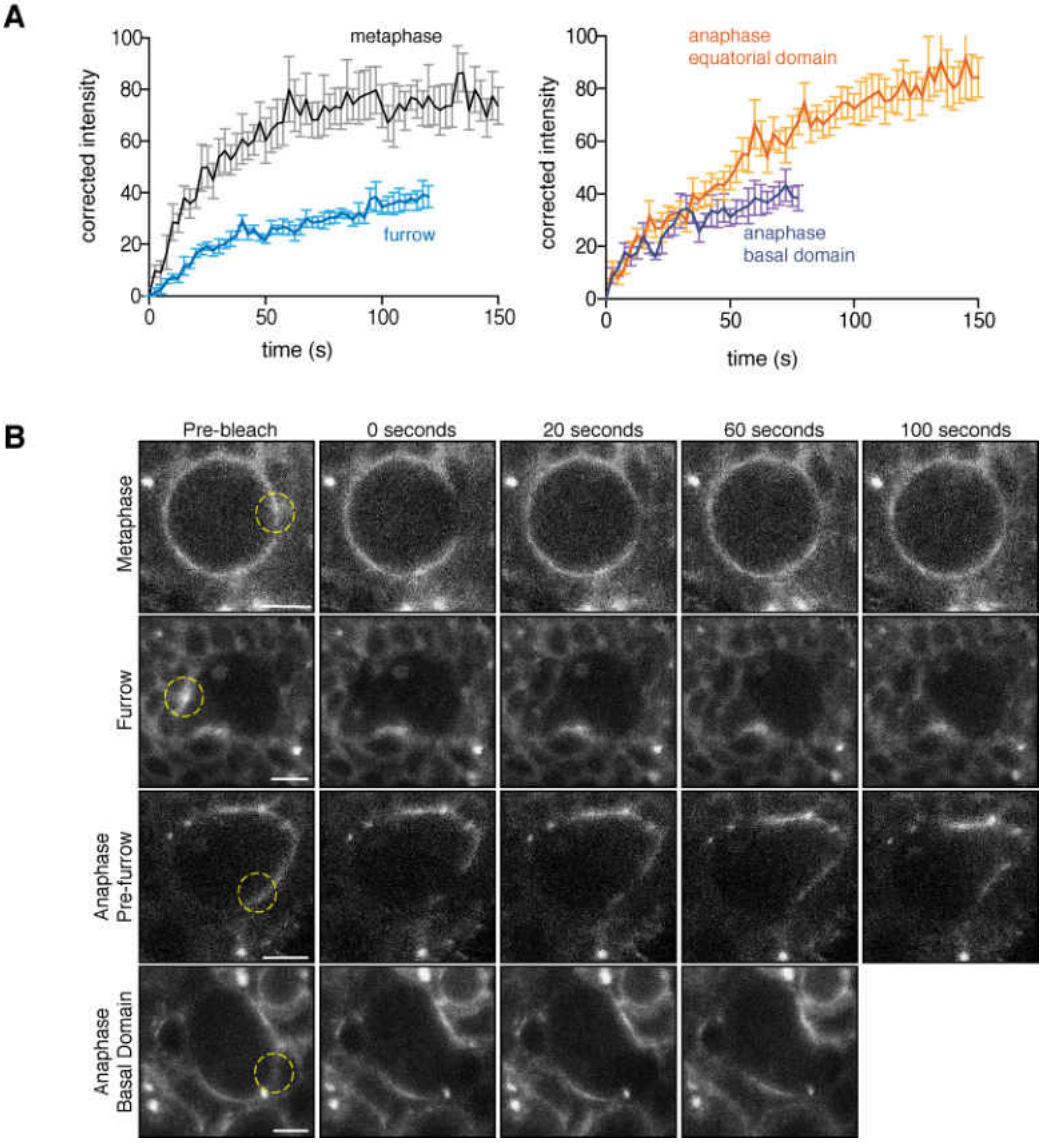

FIGURE 3.5. Dynamics of myosin during mitosis in neuroblasts. (A) Quantification of myosin recovery after photobleaching. Bleaching was performed in sqh^{ax3}; *Sqh-GFP* larval neuroblasts. The basal domain intensity was not measured for the entire time frame as the basal myosin domain depletes as mitosis proceeds. Values were normalized with intensity prior to bleaching as 100% and intensity immediately after bleaching as 0%. Basal domain data is truncated to ensure the measurements did not overlap with the depletion of basal myosin. Error bars represent 1 SD. (C) Recovery of myosin after photobleaching. Circles indicate the regions of the cortex that were bleached. Scale bars represent 10 µm.

Phospholipid binding is required for proper localization of the cleavage furrow

Interaction with the phospholipid membrane is critical for proper localization of the cleavage furrow. Anillin is known to link the actinomyosin contractile ring to the phospholipid membrane through interaction with PIP_2 and mutations in Anillin have been shown to cause mislocalization of the cleavage furrow (D'Avino et al., 2008; Liu et al., 2012). Recently it has also been shown that the human homolog of Tum (RacGAP50c) also binds to PIP_2 through its C1 domain to attach the spindle to the cortex and Tum has been shown to form complexes with anillin at the plus-ends of microtubules (Lekomtsev et al., 2012; Gregory et al., 2008). These components act together to link the cytokinetic ring, the mitotic spindle, and the cell cortex together to ensure correct localization of the cleavage furrow.

As PIP_2 has been reported to be enriched at the cleavage furrow, we expressed the a GFP fusion of the PH domain of PhospholipaseCδ1 (PH-GFP) (Liu et al., 2012; Dasgupta et al., 2009) to determine the localization of PIP_2 in neuroblasts and if it was also enriched at the basal myosin domain. PH-GFP localized to the entire cortex with enrichment at regions of cell-cell contact (Figure 3.6.A). In addition, if expression was increased further, a dominant negative phenotype was observed. In this case, the cleavage furrow begins to contract but as it contracts the furrow migrates to a new position eventually leading to equal sized daughter cells (Figure 3.6.B).

Discussion

Previously, I had proposed a model in which *Drosophila* neuroblasts produced asymmetric cytokinesis through asymmetric cortical extension (Chapter 2 of this

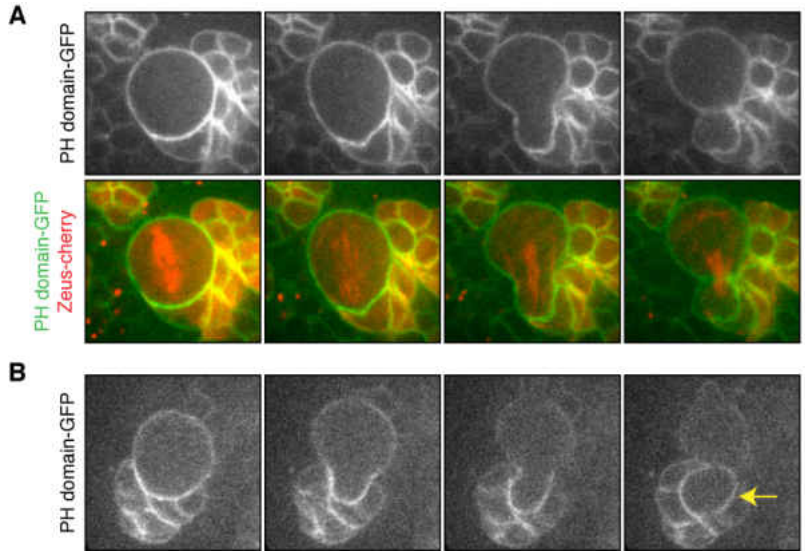

FIGURE 3.6. Interaction with PIP_2 is required for proper furrow positioning. (A) Localization of PIP_2 in larval neuroblasts. PIP_2 localization was determined using a construct of the PH domain of PhospholipaseCδ1 (PH-GFP) which binds to PIP_2. Position in the cell cycle was determined by expression of UAS-Zeus-Cherry. (B) Over-expression of PhospholipaseCδ1 PH domain causes a change in cleavage furrow position. The PH-GFP construct was expressed using *worniu*-Gal4. Images were taken 12 s apart.

work). In this chapter, I demonstrated that mutants that retain apical myosin and undergo equal cortical extension at both poles but still divide asymmetrically.

This led me to propose a new model by which neuroblasts divide asymmetrically by shifting the cleavage furrow, as in both wild-type and 14-3-3 mutants the cleavage furrow is positioned not at the spindle midzone but at a position more basal to the spindle midzone (Figure 3.7). The cleavage furrow is shifted by interactions between the mitotic spindle and a non-equatorial region of the cortex.

The basal myosin domain contracts as anaphase progresses and as the basal domain contracts it comes into contacts with the mitotic spindle at region away from the spindle midzone. We observed that midzone components such as Polo originally localize to the position of the metaphase plate but their position shifts

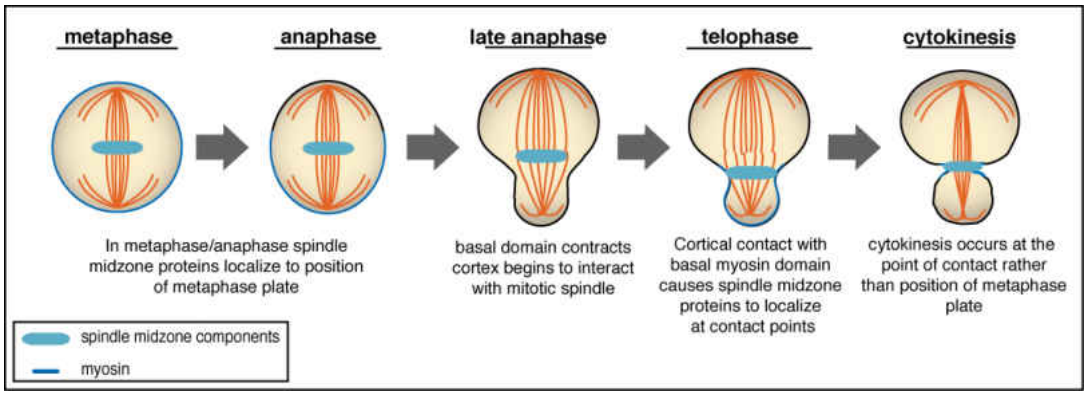

FIGURE 3.7. Model: Contraction of the basal myosin domain shifts furrow position by forming new interactions with the mitotic spindle

toward the basal pole as the basal domain contracts where their position finally correlates with the position of the cleavage furrow. Pavarotti also follows this pattern but if the basal myosin domain is lost, the shift of the midzone proteins is not seen (Cabernard et al., 2010) (Figure 4.2.B) indicating this shift is dependent on the basal myosin domain.

We propose that interaction of the cortex with the microtubules are required for the shift of the cleavage furrow. It has been demonstrated in sea urchin embryos that the cleavage furrow can be repositioned if the furrow is repositioned or new contractile regions can be created by bringing them into contact with the mitotic spindle (Bement et al., 2006). Visually, it appears that the microtubules are packed more densely at the basal pole that interacts with the cell cortex. In mutants where the interaction with between the cortex and the cleavage furrow are disrupted, such as in the case of over-expression of the PH-domain of PhospholipaseCδ1, the cleavage furrow does not form at the position more basal to the spindle. This indicates that the appropriate interaction at the position of the basal domain is required for proper furrow formation, as even when the basal domain contracts at

the same point as in wild-type it is unable to restablish the cleavage furrow at the same position as in wild-type neuroblasts.

The basal myosin domain is contractile in its own right as demonstrated by treating neuroblasts with colcemid (Cabernard et al., 2010; Connell et al., 2011). The basal domain contracts and a very small "daughter cell is formed but this is not a complete furrow as the required spindle components are not present. This also indicates the the mitotic spindle is required to form a daughter cell that is the same size as seen in wild-type. This is most likely achieved through the mitotic spindle pushing out towards the cortex counteracting the contractile force of the basal domain

C. elegans Q neuroblasts have also been shown to have a basal myosin domain although the exact mechanism has not been elucidated. The authors propose a mechanism by which the contraction of the basal domain forces the contents of the cell towards one pole similar to our model of asymmetric cortical extension (Ou et al., 2010). But could the shift in furrow position in this lineage be due to contraction of the basal domain onto the spindle as in *Drosophila* neuroblasts?

Materials & Methods

Fly lines

All mutant chromosomes were balanced over CyO actin:GFP, TM3 actin:GFP, Ser, e, or TM6B Tb. We used Oregon R as wild type and the following mutant chromosomes and fly strains: *UAS-14-3-3ζ-RNAi* (104496KK), *UAS-14-3-3ε-RNAi* (108129KK), *UAS-Gβ13F-RNAi* (100011KK) and *UAS-Rho-RNAi*(10942KK) (Dietzl et al., 2007); *worGal4, Sqh-GFP, UAS-zeus-cherry* (Cabernard et al., 2010), *UAS-PLCδ1PH-GFP* (Dasgupta et al., 2009), sqh^{ax3}*; Sqh-*

GFP (Royou et al., 2004), *Polo-GFP* (Moutinho-Santos et al., 1999). For RNAi and PH domain-GFP crosses, larva were collected at room temperature for 48 hours and then shifted to 25 °C for approximately 48 hours prior to imaging.

Live imaging

Second or third larval brains were prepared for imaging as previously described (Siller et al., 2005). Five to nine Z steps were collected at 1- to 2 µm intervals every 12 s. Live imaging was performed using a spinning disk confocal microscope equipped with a Hamamatsu EM-CCD camera (Hamamatsu, Japan) using a 63 x 1.4 numerical aperture oil immersion objective. Pixel intensity measurements were performed using ImageJ (National Institutes of Health, Bethesda, MD).

Photobleaching

Second instar larval brains from sqh^{ax3}*; Sqh-GFP* larva were prepared as previously described (Siller et al., 2005). FRAP experiments were performed using a Leica SP2 confocal microscope. Bleaching was performed using the FRAP module of the Leica SP2 Confocal Software. Prior to bleaching 3 frames were taken 0.865 seconds apart and post bleaching 120 frames were taken 2.5 seconds apart. Bleaching conditions were 20 scans, each taking 0.865 seconds at 100% laser power. Intensity measurements were performed using ImageJ. Myosin intensity was determined at the middle of each bleached spot. General photobleaching was determined using a distant region of the brain as described in Shen et al. (2008).

Bridge to Chapter IV

Chapter III proposed a revised model for the mechanism by which the basal myosin domain acts. I showed that restricting extension at the apical pole still leads to asymmetric cytokinesis, which does not support the model presented in Chapter II. I propose that the basal domain acts to reposition the cleavage furrow by contracting which brings the cortex in contact with the mitotic spindle but this contractile region is positioned nearer the basal pole of the cell leading to the asymmetry in daughter cell size. In Chapter IV, I investigated the pathway that regulates the basal domain. Data in Chapter II suggested that Gβ13F could be a regulatory molecule so we tested that hypothesis and investigated if there were similarities between the regulatory pathway for the cleavage furrow and that of the basal myosin domain.

CHAPTER IV

Gβ13F PROMOTES BASAL MYOSIN LOCALIZATION NECESSARY FOR ASYMMETRIC CELL DIVISION OF *DROSOPHILA* NEUROBLASTS

Introduction

During development, stem cells often divide to create daughter cells of different sizes which is thought to be necessary so that the growth time between stem cell divisions is minimized (Joregensen and Tyers, 2004). Asymmetric cytokinesis is used to create daughter cells of different sizes during mitosis by effectively biasing the spindle towards one pole of the cell (Glotzer, 2004). In most systems the mother cell is large and the spindle can be biased towards one pole prior to the onset of anaphase. If the mother cell is smaller, such as in the *Drosophila* neuroblasts, the mitotic spindle localizes to the center of the cell as the cell is not large enough to accommodate a biased spindle. Recently, it has been shown in several systems, the *Drosophila* neuroblasts and the *C. elegans* Q neuroblasts that a polarized myosin domain is required for asymmetric cytokinesis (Cabernard et al., 2010; Ou et al., 2010).

Drosophila neuroblasts divide asymmetrically to form a large, apical, self-renewing neuroblasts and a smaller, basal, differentiating ganglion mother cell (GMC) (Knoblich, 2008). The polarized myosin domain (located basally in *Drosophila* has been shown to act as a restrictive force and it has been hypothesized that the restriction at the basal pole causes an extension at the opposing pole and it is this asymmetric extension that effectively relocalizes the mitotic spindle so that it is biased towards one pole (Connell et al., 2011).

During mitosis in neuroblasts, myosin is localized to the entire cortex during metaphase but upon the onset of anaphase myosin depletes from the apical pole and is retained at the basal pole (in symmetric cell division, myosin is lost from both poles) (Green et al., 2012). Pavarotti (Zen4 in C. elegans) and Anillin are also retained at the basal pole during anaphase, although their role in the process of asymmetric cytokinesis is unclear (Cabernard et al., 2010). The common thread between these proteins is that they are all involved in producing the contractile forces needed at the cleavage furrow for cytokinesis. Although they are co-localized they might not be dependent on one another for localization at the basal pole as it has been shown that Pavarotti is not required for basal myosin localization (Cabernard et al., 2010).

The exact pathway that establishes the localization of basal myosin domain of *Drosophila* has not been fully elucidated. It has been determined to be regulated by Pins, a component of the pathway that aligns the mitotic spindle to the correct axis during asymmetric cell division (Cabernard et al., 2010). In neuroblasts, Pins binds to Gαi at the apical pole dissociating it from G$\beta\gamma$, activating both G$\beta\gamma$ and Gαi proteins so they can act in other pathways (Yu et al., 2000) . Gαi mutants do not lose basal myosin at anaphase showing it is not required for the asymmetric localization of myosin (Cabernard et al, 2010). Previous work has not directly tested the involvement of G$\beta\gamma$, but overexpression of Gαi leads to the loss of basal myosin and the neuroblasts divide symmetrically (Schaefer et al., 2001; Connell et al., 2011). This suggests that G$\beta\gamma$ is involved as excess Gαi could bind to free G$\beta\gamma$, sequestering it from its signaling pathway. This is further supported by the fact that overexpression of constitvely active Gαi (a form that does not bind G$\beta\gamma$) does not cause loss of the basal domain. In addition it is known that mutants of

the Gβ subunit, Gβ13F, have neuroblasts that divide symmetrically which could be due to a failure in asymmetric contractile furrow positioning (Fuse et al., 2003). Here we examined the role that Gβ13F plays in the regulation of the basal myosin domain in Drosophila neuroblasts.

Results

Gβ13F is required for basal localization of Myosin during anaphase in larval neuroblasts

We determined if Gβ13F is required for the localization of myosin to the basal domain in anaphase during larval neuroblasts asymmetric cell division. Gβ13F mutants die in embryonic stages so we used Gβ13F RNAi driven by a neuroblast-specific driver, worniu-Gal4 line. We visualized myosin localization by expressing a GFP-tagged construct of the myosin II regulatory light chain (Spaghetti squash in Drosophila, Sqh-GFP). We found that myosin depletes from the basal pole during anaphase in Gβ13F knockdown brains and these neuroblasts were much smaller than those seen in wild-type brains (Figure 4.1.A). Comparing the daughter size ratios to wild-type neuroblasts and symmetrically dividing cells, we found that 87.5% of Gβ13F RNAi divisions were symmetric (Figure 4.1.B, C). We classified symmetric divisions as those that had a daughter size ratio of approximately one.

Gβ13F is required for the basal localization of the furrow components, Anillin and Pavarotti

It has previously been shown that other furrow components, Pavarotti and Anillin (Flybase: Scraps), have the same localization pattern as myosin during mitosis in wild-type neuroblasts (Cabernard et al., 2010). To determine if their

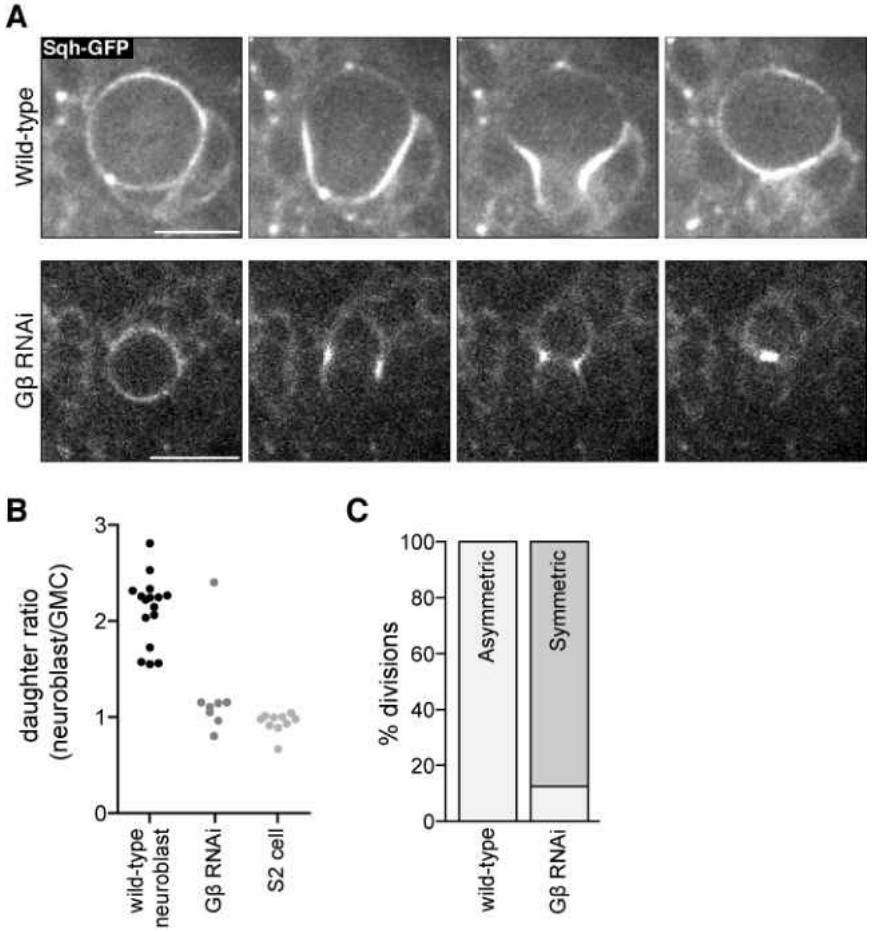

FIGURE 4.1. Gβ13F is required for basal myosin localization. (A) Myosin II (Sqh-GFP) localization in wild-type and Gβ13F RNAi larval neuroblasts. RNAi constructs were expressed using *worniu*-Gal4. Images were taken 12 s apart. Scale bar, 10 µm. (B) Daughter size ratio in wild-type neuroblasts, Gβ13F RNAi neuroblasts, and *Drosophila* S2 cells (symmetrically dividing cells). Ratio was determined by measuring the diameter of both daughter cells. (C) Quantification of Gβ13F RNAi phenotype in comparison wild-type. Light grey bars represent the percent of asymmetric divisions while dark grey bars represent the percent of symmetric divisions. The symmetry of division was determined by looking at the daughter cell ratios in Panel B, with those being have a daughter size ratio of approximately 1 being considered symmetric divisions

localization is also regulated by Gβ13F, we expressed Gβ13F RNAi in neuroblasts expressing GFP-tagged constructs of either Anillin (Anillin-GFP) or Pavarotti (Pav-GFP). In mutant neuroblasts expressing Anillin-GFP, Anillin was not retained at the basal pole at anaphase onset, mimicking the pattern seen with myosin

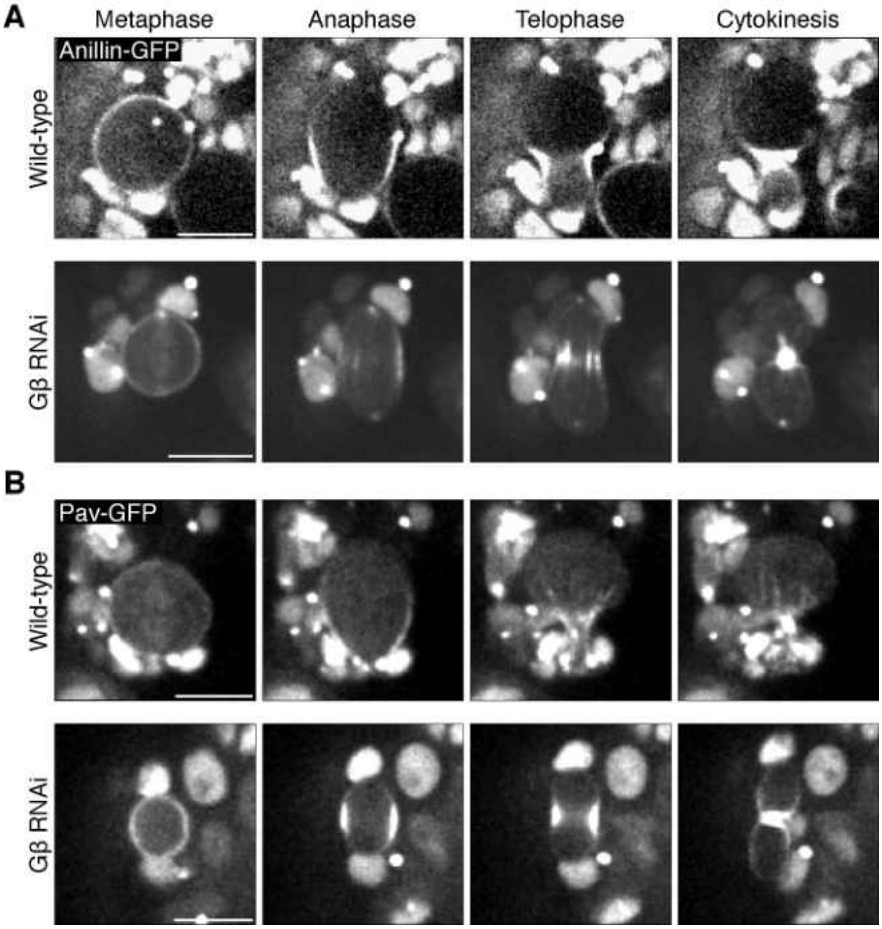

FIGURE 4.2. Gβ13F is required for the basal localization of Anillin and Pavarotti. (A) Anillin (Anillin-GFP) localization in wild-type and Gβ13F RNAi larval neuroblasts. Anillin-GFP and RNAi constructs were driven using *worniu*-Gal4. Images were taken 12 s apart. Scale bar, 10 µm (B) Pavarotti (Pav-GFP) localization in wild-type and Gβ13F RNAi larval neuroblasts. Pav-GFP and RNAi constructs were driven using *worniu*-Gal4. Images were taken 12 s apart. Scale bar, 10 µm

(Figure 4.2.A). Similarly, Pav-GFP was also found to deplete from the basal pole at anaphase onset in the Gβ13F RNAi background (Figure 4.2.B). We conclude that Gβ13F is required for the basal localization of all furrow components known to localize to the basal domain.

Anillin is not required for basal myosin localization

Anillin and Pavarotti colocalize with myosin to the basal domain of neuroblasts during cytokinesis (Cabernard et al., 2010). It has been previously shown that Pavarotti is not required for basal myosin localization, but the role of anillin has not been determined. Anillin is not required for myosin localization at the cleavage furrow although it is required for proper furrow localization (Piekny and Glotzer, 2008). We investigated if Anillin is required for basal myosin localization by expressing Anillin RNAi in neuroblasts using the worniu-Gal4 driver. Loss of Anillin did not affect myosin localization to the basal domain showing that Anillin is not required for basal myosin localization (Figure 4.3.A). Although basal localization was not affected there were several phenotypes seen in relation to the cleavage furrow (Figure 4.3.A, B). These phenotypes were that the cleavage furrow failed to form there were two furrows formed, or the furrow migrates to a new position. In the case of the furrow migrating, it begins at the appropriate location but as it contracts the furrow moves to a new position and this often leads to equal-sized daughter cells. Anillin and Pavarotti also co-localize at the basal domain and we tested in Anillin is required for the localization of Pavarotti to the basal domain. Pav-GFP localizes to the basal domain in Anillin RNAi neuroblasts and have similar cleavage furrow phenotypes as seen with Myosin-GFP (Figure 4.3.C). These data show that Anillin is not required for the basal localization of Myosin or Pavarotti in neuroblasts.

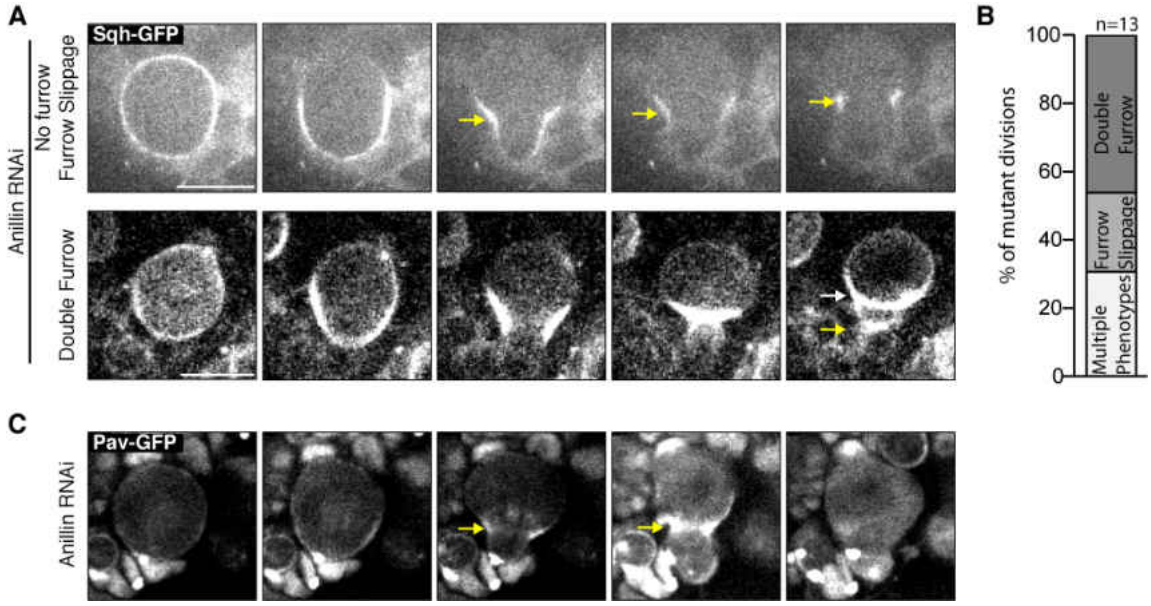

FIGURE 4.3. Anillin is not required for basal myosin localization. (A) Myosin (Sqh-GFP) localization in Anillin RNAi neuroblats. The upper panel depicts a neuroblast that never furrowed and the furrow slipped to a new position (yellow arrow indicates position of cleavage furrow). The bottom panel depicts a phenotype where a double furrow formed (yellow arrow indicates original furrow and white arrow indicates second furrow). Anillin RNAi was expressed in neuroblasts under the control of *worniu*-Gal4. Images were taken 12 s apart. Scale bar, 10 µm (B) Quantification of Anillin RNAi phenotypes. Multiple phenotypes consists of either the double furrow or furrow slippage phenotype plus the incomplete furrow phenotype. (C) Pavarotti (Pav-GFP) localization in Anillin RNAi neuroblasts. This panel depicts a neuroblast that demonstrates "furrow slippage" (daughter cell grows larger) and the furrow does not resolve. Anillin RNAi and Pav-GFP were expressed using *worniu*-Gal4.

Actin localizes to the entire cortex during cytokinesis and is not required for basal myosin localization

Actin is a major component of the cytoskeleton and forms a complex with myosin to produce contractile forces in the cell, so we determined if Actin is polarized to the basal domain during cytokinesis similar to myosin. We stained wild-type neuroblasts with phalloidin to determine actin localization. We found that actin localized to the entire cortex (Figure 4.4.A). There appeared to be an enrichment at the apical pole during metaphase with the average ratio of apical to

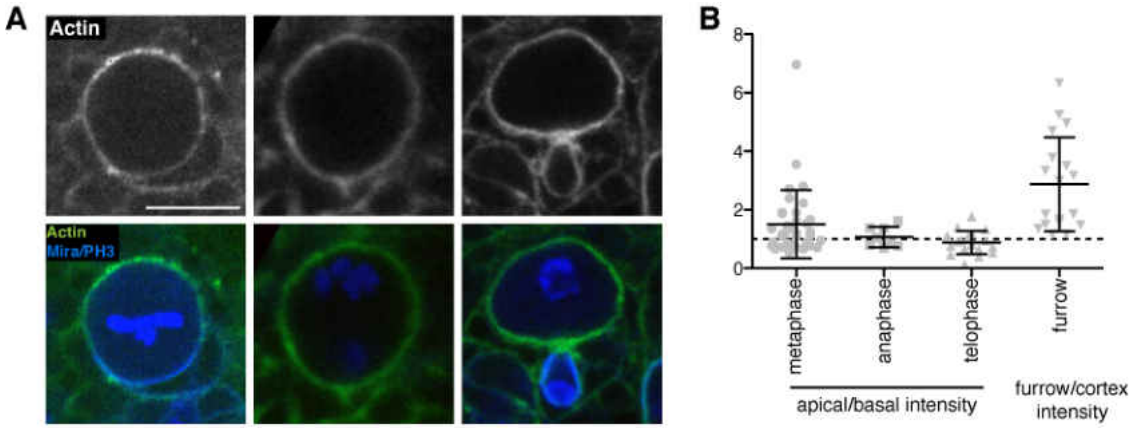

FIGURE 4.4. Actin is apically enriched at metaphase. (A) Actin localization in third instar larval neuroblasts. Actin localization was visualized using 555-Phalloidin. Cell cycle was determined using Phosphohistone 3 (PH3) and Miranda marks the basal pole. (B) Quantification of actin enrichment at the apical versus basal pole. The average cortical intensity at the apical pole was compared to the average cortical intensity at the basal pole. The enrichment of the furrow was determined by comparing the average intensity of the furrow to the average of the apical and basal cortical intensities. The dashed line marks the ratio if the intensity at both poles were equal. Error bar, 1 S.D.

basal intensity being 1.509 ± 1.167 (Figure 4.4.B). During anaphase and telophase the two poles have similar intensities with ratios of 1.069 ± 0.3509 and 0.8858 ± 0.3965 respectively. The cleavage furrow was enriched when compared to the rest of the cortex with a furrow to cortex ratio of 2.875 ± 1.610.

Although actin is not polarized to the basal pole, actin could be required for basal myosin localization. It is known that actin is required for myosin polarization to the apical pole of mesodermal precursor cells in ventral furrow formation during gastrulation in Drosophila embryos (Dawes-Hoang et al., 2005). In the case of the cleavage furrow though, initial localization of myosin does not require actin but maintenance of actin at the cleavage furrow does (Dean et al., 2005). To test if actin is required for myosin localization to the basal domain we treated neuroblasts with LatrunculinA, which causes depolymerization of actin filaments. In controls treated with DMSO alone, myosin localized to the basal myosin domain as in wild-

type neuroblasts (Figure 4.5.A). In addition, upon treatment with LatA, myosin still localized to the basal domain although it did not appear as smooth as in wild-type. Consistent with the loss of actin, the neuroblasts treated with LatA showed cytokinesis failure and did not have any contractile activity at all. We conclude that actin is not required for the localization of myosin to the basal domain. We also looked at the role of the formin, Diaphanous, which acts in the formation of actin filaments and is required for myosin maintenance but not recruitment to the furrow (Dean et al., 2005; Watanabe et al., 2008) and found that it as not required for myosin localization at the basal pole (Supplemental Figure A.3).

To further confirm that actin is not required for basal myosin localization, we visualized the localization of a dominant-negative form of the myosin II heavy chain (Zipper (Zip) in *Drosophila*). The dominant-negative Zipper (Zip^{DN}) allele has the actin binding domain of myosin replaced by YFP and can no longer bind to actin. Wild-type Zipper shows a localization pattern similar to that seen when visualizing sqh-GFP, although the construct tends to form clumps that are not seen with Sqh-GFP. Zip^{DN} remains localized to the basal domain at anaphase onset, confirming that actin is not required for myosin localization to the basal domain in *Drosophila* neuroblasts (Figure 4.5.B).

Discussion

Drosophila neuroblasts produce daughter cells of different sizes through a mechanism that takes advantage of a polarized myosin domain. Previously work had shown that Pins is required for basal myosin localization but its binding partner Gαi is not (Cabernard et al., 2010). When Pins binds Gαi, it also activates G$\beta\gamma$ so we investigated the role of Gβ13F in the formation of the polarized myosin

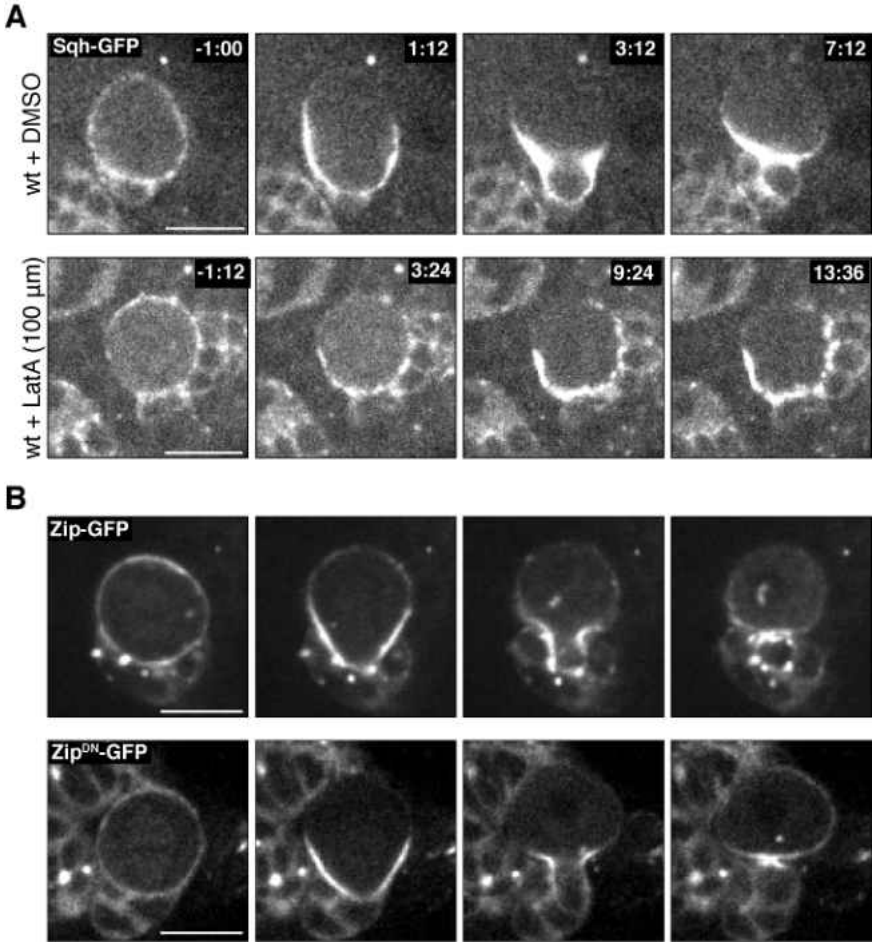

FIGURE 4.5. Actin is not required for basal myosin localization. (A) Myosin (Sqh-GFP) localization in DMSO control and Lat-A treated larval neuroblasts. Neuroblasts were treated with 100 µM LatA and imaged immediately after treatment. Images were taken 12 s apart. Time 0 = anaphase start. Scale bar, 10 µm. (B) Localization of wild-type (Zip-GFP) and mutant (Zip^{DN}-YFP) myosin constructs. Zip^{DN}-YFP has the actin binding domain replaced with YFP and can no longer bind actin. Both Zip-GFP and Zip^{DN}-YFP constructs were expressed using *worniu*-Gal4. Images were taken 12 s apart. Scale bar, 10 µm.

domain. RNAi knockdown of Gβ13F caused loss of basal myosin localization and led to symmetric divisions.

In addition, Gβ13F knockdown also led to the loss of Pavarotti and Anillin which also localize in a similar pattern to myosin. Interestingly, it has been shown that Pav (Cabernard et al., 2010) and Anillin (this work) are not required for

basal myosin localization. leading to the question of what is their role at the basal myosin domain? One possibility is that the mechanism that clears them from the polar regions is the same as the mechanism that clears polar myosin and they are retained as a byproduct of retaining myosin. Another possibility is that they have a function in the formation of the cleavage furrow at the more basal position similar to the function of equatorial Pavarotti and Anillin in symmetrically dividing cells.

Gβ13F localization and it has Gβ13F localizes to the entire cortex of the neuroblasts unlike Gαi and Pins, which are polarized to the apical pole (Fuse et al., 2003). As Pins is required for basal myosin localization, it supports the idea that it is the apical population of Gβ13F that is required for basal myosin localization. One main question that needs to be further investigated is how the signal at the apical pole is translated to the retention of myosin at the basal pole?

Anillin also localizes to the basal domain during asymmetric cytokinesis and loss of Anillin results in multiple phenotypes. Several of the phenotypes have been previously reported such as the lack of furrowing and the migration of the furrow to a new position. We have observed a new phenotype where the normal cleavage furrow forms and constricts completely and then a second furrow forms and contracts completely but it appears to retract before abscission. In our system the second furrow typically forms biased towards the apical pole of the cell near the location of the metaphase plate. This second furrow could unique to *Drosophila* neuroblasts as a result of shifting the furrow to a more basal position. Perhaps in Anillin mutants, the equatorial myosin is still able to contract and this contraction produces the second furrow.

We attempted to work backwards from myosin to Gβ13F, by looking at Rho mutants as Rho is an important regulator of myosin during mitosis: being

required for myosin localization at the entire cortex during early mitosis and also for localization of myosin to the equatorial region during late mitosis (O'Connell et al., 1999). Rho RNAi neuroblasts did not have a basal myosin domain but myosin was lost from the entire cortex throughout mitosis (Supplemental Figure A.4). From this data, we cannot conclude if Rho acts on myosin downstream of Gβ13F. It is possible that Rho is required to get myosin to the cortex during metaphase but that another factor downstream of Gβ13F acts during anaphase to retain basal myosin. It is also possible that Rho itself is the factor downstream of Gβ13F but these possibilities cannot be teased apart from this data. Future work will be directed towards determining the factors downstream of Gβ13F that are required for basal myosin localization.

Materials & Methods

Fly lines

All mutant chromosomes were balanced over Cyo actin-GFP, Ser, TM6B TB, or TM3 Ser actin-GFP. We used Oregon R as wild-type and the following mutant chromosomes and fly strains: *worGal4, Sqh-GFP, UAS-zeus-cherry* (Cabernard et al., 2010); *wor-Gal4, UAS-zeus-cherry* (Cabernard and Doe, 2009); *anillin-GFP* (Silverman-Gavrilla et al., 2008); *wor-G4, UAS-GFP-PavNLS5* (Minestrini et al., 2003); *UAS- Gβ13F-RNAi* (100011KK), *UAS-Anillin-RNAi* (33465GD), and *UAS-Rho-RNAi* (10942KK) (Dietzl et al., 2007); and *UAS-Zip-GFP* and *UAS-ZipDN-YFP* (Dawes-Hoang et al., 2005) (Kindly provided by E. Wieschaus). For Zip-GFP and Zip^{DN}-YFP crosses were performed at 25 °C. For RNAi crosses, larva were collected at room temperature for 48 hours and then shifted to 25 °C (Rho

and Anillin RNAi) or 30 °C (Gβ13F RNAi) for approximately 48 hours prior to imaging.

Live imaging

Second or third larval brains were prepared for imaging as previously described (Siller et al., 2005). Five to nine Z steps were collected at 1- to 2-µm intervals every 12 s. Live imaging was performed using a spinning disk confocal microscope equipped with a Hamamatsu EM-CCD camera (Hamamatsu, Japan) using a 63 x 1.4 numerical aperture oil immersion objective. Pixel intensity measurements were performed using ImageJ (National Institutes of Health, Bethesda, MD). LatrunculinA treatment was performed on the strain *+; worGal4, UAS-Cherry:Jupiter, Sqh:GFP*, using a final concentration of 100 µM, with live imaging beginning immediately after treatment.

Immunohistochemisty

We fixed and stained wild-type larval brains as described previously (Siegrist & Doe, 2006). Primary antibodies used were rabbit α-phosphohistone H3 (1:10,000; Santa Cruz Biotechnology), guinea pig α-Mira (1:500, Kindly provided by C.Q. Doe), rabbit α-Gβ13F (1:500, Kindly provided by F. Matsuzaki)(Fuse et al., 2003), rat α-Par6 (1:250), and Alexa Fluor 555-phalloidin (1:500, Life Technologies). Confocal images were acquired on a BioRad Radiance 2000 laser scanning confocal microscope equipped with a 60x 1.4 NA oil-immersion objective using Lasersharp software with a Kalman average of 3. Pixel intensity was measured in ImageJ.

Cell culture

Drosophila S2 cells were maintained at room temperature in Schneiders medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (Sigma-Aldrich). Cells were transiently transfected with pMT-Zeus-Cherry with Effectene (Qiagen, Valencia, CA) according to the manufacturers instructions, and expression was induced with 0.5 M copper sulfate 20 hr prior to imaging. Immediately before imaging, cells were resuspended in Schneiders medium supplemented with 10% fetal bovine serum.

Bridge to Chapter V

Chapter IV discussed the pathway by which the basal myosin domain is regulated. I showed that Gβ13F is required for the localization of the basal furrow components, including Pavarotti and Anillin. I also investigated if there were similarities between the pathway that regulates the cleavage furrow and that which regulates the basal myosin domain. I showed that actin and other related proteins show a similar requirement as at the cleavage furrow, although results for other proteins were ambiguous. In Chapter V, I will discuss the findings presented in this dissertation and how they contribute to the overall picture of how cells are able to produce daughter cells of different sizes. I will also discuss potential areas of investigation based on these results.

CHAPTER V

CONCLUDING REMARKS

Summary

My thesis work focused on asymmetric cytokinesis in *Drosophila* neuroblasts and the role of the basal myosin domain in this process. I tested several models of how the basal myosin domain achieves asymmetric cytokinesis and also worked to determine the pathway regulating the basal myosin domain. I first tested the model that the basal myosin domain functions to restrict cortical extension at the basal pole while allowing the apical pole to extend. This would ultimately lead to asymmetric cytokinesis by effectively positioning the metaphase plate/spindle midzone closer to one pole than the other. Looking at mutants that lose myosin from both poles supported this model as they exhibited equal cortical extension (loss of restriction at basal pole) and this correlated to symmetric cytokinesis. The discovery of mutants that retained apical myosin allowed me to test this model further. These mutants divided asymmetrically although they exhibited equal cortical extension at both poles. Based on observations of the interaction of the mitotic spindle with the cortex, we propose a model that asymmetric cytokinesis is produced by the contraction of the basal myosin domain onto the mitotic spindle. The interaction of the mitotic spindle with this region of the cortex positioned more basally than the metaphase plate shifts the furrow toward a more basal position.

I demonstrated that the basal myosin domain is regulated by G protein signaling, specifically through the G$\beta\gamma$ subunit. This was shown through knocking down Gβ13F activity in neuroblasts. In addition to regulating basal myosin,

Gβ13F is also required for the basal localization of Pav and Anillin. Although Anillin, Pav, and Myosin are all colocalized, Anillin was found to not be required for the localization of either pav or myosin. As a pathway between G$\beta\gamma$ has not been elucidated in neuroblasts, I also investigated if the basal domain is regulated similarly to the cleavage furrow pathway which is contiguous with the basal myosin domain. Photobleaching data indicates that these two domains are regulated differently temporally as the equatorial domain recovers more quickly than the basal myosin domain. This suggests that equatorial myosin is not as stable a structure as the basal domain in this time frame. Investigating components of the furrow pathway showed that most of the components (including furrow components, Pav and Anillin) are not required for basal myosin localization. Components of the actin pathway did affect contractile activity but not localization. The only furrow pathway component that affected myosin localization was Rho, but it affected cortical localization during metaphase so it is unclear if it acts downstream of G$\beta\gamma$.

Future Considerations

The data presented in Chapter III demonstrates that apical myosin is retained in 14-3-3 mutants and this raises several important questions. Are these proteins responsible for polar depletion in symmetrically dividing cells (would myosin be retained at both poles in 14-3-3 mutants)? And how does this fit into a pathway with Gβ? One could think of two possible models: Gβ inhibits 14-3-3 at the basal pole to cause retention of basal myosin or Gβ and 14-3-3 are in separate pathways with 14-3-3 causing depletion and Gβ reestablishing myosin at the basal domain concurrently (Figure 5.1). Although differentiating between the two models could

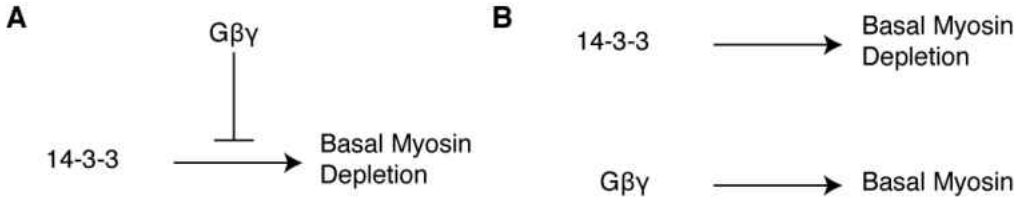

FIGURE 5.1. Possible Mechanisms for Gβ13F Regulation of Aurora B. (A) Aurora B causes basal myosin depletion but this pathway is inhibited by Gβ activity. (B) Gβ and Aurora B act in separate pathways to reestablish myosin localization and cause myosin depletion at the basal pole respectively.

be difficult as epistatic analysis could produce the same result from both pathways. There does appear to be an additional positive signal required if Gβ is inhibiting 14-3-3 as the myosin domain must have a contractile function.

Chapter IV demonstrates that Gβ13F is required for the localization of myosin to the basal domain although the downstream factors are not known. Interactions between G$\beta\gamma$ and 14-3-3 have not been reported. A common downstream effectors of G$\beta\gamma$ are PAK kinases (Leberer et al., 2000; Leeuw et al., 1998), which are known to phosphorylate the regulatory light chain of myosin (Goeckeler et al., 2000; Chu et al., 2000). To determine if a PAK kinase is involved, a directed screen could be performed.

It has been found that Pav and Anillin are also localized to the basal domain (Cabernard et al., 2010). But using RNAi to knockdown either of them does not affect myosin localization, so that leads to the question of why they are also basally localized. Loss of G$\beta\gamma$ causes loss of these proteins also so it is possible that the pathway that regulates polar depletion of myosin also regulates the polar depletion of Pav and Anillin. In this situation, the retention of Pav and Anillin could be a byproduct of the retention of myosin. Another possibility is that they are localized to the basal domain to ensure proper formation of the cleavage furrow upon interaction of the cortex with the mitotic spindle.

A larger question is what is the role of asymmetric cytokinesis during development. Theoretically the creation of daughter cells could be done with symmetric cytokinesis as long as the fate determinants were segregated properly. One hypothesis is that the retention of size allows the neuroblast to divide more quickly (Joregensen and Tyers, 2004). *Drosophila* neuroblasts divide very regularly with one hour between divisions and cells must grow to a certain size prior to being able to divide again. Neuroblasts retain ∼90% of their size (unpublished data). One difficulty in testing this in neuroblasts is that the daughter cells must retain their appropriate identities or the rate of divisions could be affected. Many mutants that cause symmetric divisions also cause misalignment of the spindle which would affect the segregation of fate determinants. Gβ mutants might be able to help answer this question as it has been previously reported that there are no spindle alignment defects and the fate determinants are appropriately segregated (Yu et al., 2000; Schaefer et al., 2001; Fuse et al., 2003). Unpublished data shows that Gβ mutant neuroblasts do not fully regain their size as they tend to become smaller over time whereas wild-type neuroblasts maintain their size over multiple divisions.

Concluding Remarks

My thesis research has identified a mechanism by which the basal myosin domain in *Drosophila* neuroblasts produces asymmetric cytokinesis. In addition, I have shown that Gβ13F is required for basal myosin localization and I have ruled out regulation of the basal domain by many of the components of the myosin regulatory pathway found at the cleavage furrow. This is interesting because the basal domain and the equatorial domain appear contiguous. These findings are the first to elucidate the mechanism by which the basal myosin domain of *Drosophila*

neuroblasts act to produce asymmetric cytokinesis and should provide clues for future studies into how the pathway is regulated. They also show a mechanism by which cells that are unable to asymmetrically position their mitotic spindle can produce asymmetric cytokinesis.

APPENDIX

SUPPLEMENTAL FIGURES

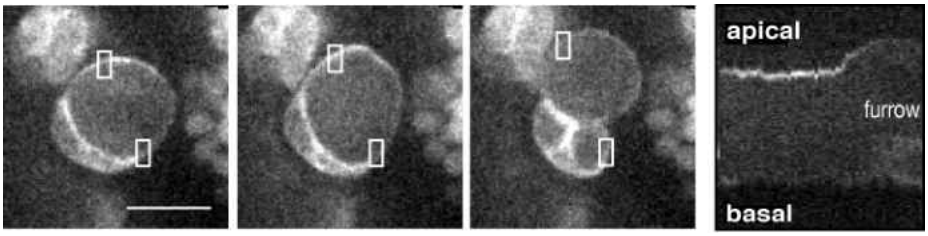

FIGURE A.1. Kymograph of a Dlg-GFP neuroblast in a region that is not contacting another Dlg-GFP cell. The apical pole shows a similar pattern as Figure 2.1.B, but the basal pole shows no significant Dlg signal at any point in the cell cycle. The images were taken at 6 second intervals, with the boxes denoting the cortical regions contained in the kymograph.

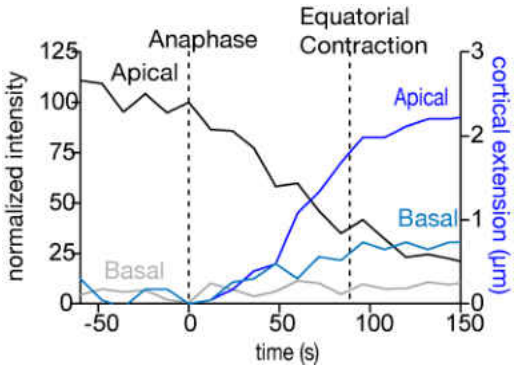

FIGURE A.2. Time dependence of cortical myosin signal and cortical position for wild-type neuroblasts expressing Dlg-GFP. Blue lines indicate the cortical position at each pole relative to the position at anaphase start. Dark lines indicate the intensity at each pole relative to the apical cortical intensity at anaphase start (determined as in Figure 2.2.C). Equatorial contraction indicates the time point at which the initiation of furrow ingression was observed.

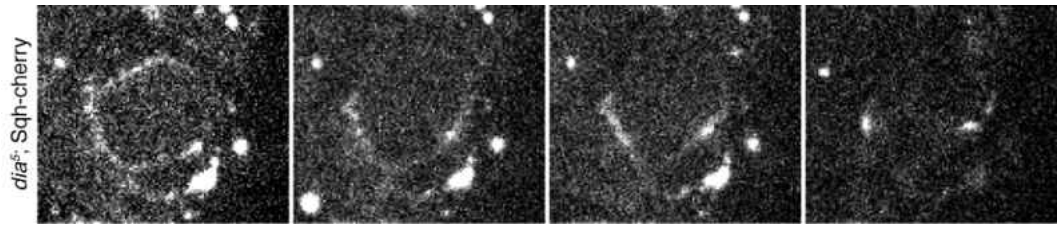

FIGURE A.3. Dia is not required for basal myosin localization. Sqh-cherry was expressed in *dia*^{5} mutants. Myosin depleted from the apical pole and remained at the basal pole and the cells did not complete cytokinesis. Images were taken every 12 seconds.

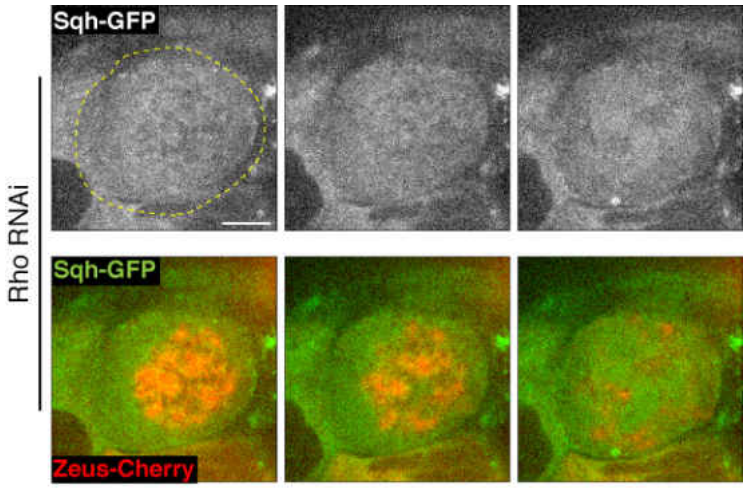

FIGURE A.4. Rho is required for cortical myosin localization. Rho RNAi expressed under the control of the *worniu*-Gal4 driver in neuroblasts. Myosin localization at the cortex was lost through the entire cell cycle. Images were taken every 12 s. Yellow dashed line indicates the perimeter of the neuroblast. Scale bar, 10 µm

REFERENCES CITED

Adam, J., Pringle, J., and Peifer, M. (2000). Evidence for functional differentiation among Drosophila septins in cytokinesis and cellularization. *Mol Biol Cell*, 11:2123–3135.

Albertson, D. (1984). Formation of the first cleavage furrow in nematode embryos. *Dev Biol*, 101:61–72.

Albertson, R. and Doe, C. (2003). Dlg, Scrib, and Lgl regulate neuroblast cell size and mitotic spindle asymmetry. *Nat Cell Biol*, 5:166–170.

Atwood, S., Chabu, C., Penkert, R., Doe, C., and Prehoda, K. (2007). Cdc42 acts downstream of Bazooka to regulate neuroblast polarity through Par-6 aPKC. *J Cell Sci*, 120:3200–3206.

Basto, R., Gomes, R., and Karess, R. (2000). Rough Deal and ZW10 are required for the metaphase checkpoint in Drosophila. *Nat Cell Biol*, 2:939–943.

Basto, R., Lau, J., Vinogradova, T., Gardiol, A., Woods, C., Khodiakov, A., and Raff, J. (2006). Flies without centrioles. *Cell*, 125:1375–1386.

Bement, W., Miller, A., and von Dassow, G. (2006). Rho GTPase activity zones and transient contractile arrays. *Bioessays*, 28:983–993.

Bonaccorsi, S., Giansanti, M., and Gatti, M. (1998). Spindle self-organization and cytokinesis during male meiosis in asterless mutants of Drosophila melanogaster. *J Cell Biol*, 142:751–761.

Bonaccorsi, S., Giansanti, M., and Gatti, M. (1999). Spindle assembly in Drosophila neuroblasts and ganglion mother cells. *Nature Cell Biol*, 2:54–56.

Bowman, S., Neumüller, R., Novatchkova, M., Du, Q., and Knoblich, J. (2006). The Drosophila NuMA homolog Mud regulates spindle orientation in asymmetric cell division. *Dev Cell*, 10:731–742.

Bringmann, H. and Hyman, A. (2005). A cytokinesis furrow is positioned by two consecutive signals. *Nature*, 436:731–734.

Brinkley, B., Stubblefield, E., and Hsu, T. (1967). The effect of Colcemid inhibition and reversal on the fine structure of the mitotic apparatus of Chinese hamster cells in vitro. *J Ultrastruct Res*, 19:1.

Cabernard, C. and Doe, C. (2009). Apical/basal spindle orientation is required for neuroblast homeostasis and neuronal differentiation in Drosophila. *Dev Cell*, 17:134–141.

Cabernard, C., Prehoda, K., and Doe, C. (2010). A spindle-independent cleavage furrow positioning pathway. *Nature*, 467:91–94.

Cai, Y., Yu, F., Lin, S., Chia, W., and Yang, X. (2003). Apical complex genes control mitotic spindle geometry and relative size of daughter cells in Drosophila neuroblast and pI asymmetric divisions. *Cell*, 112:51–62.

Chan, G., Jablonskit, S., Starr, D., Goldberg, M., and Yen, T. (2000). Human ZW10 and Rod kinetochore proteins are novel components of the mitotic checkpoint. *Nat Cell Biol*, 2:944–947.

Chu, J., Pham, N., Olate, N., Kislitsyna, K., Day, M., LeTourneau, P., Kots, A., Stewart, R., Laine, G., Jr., C. C., and Uray, K. (2000). Biphasic regulation of myosin light chain phosphorylation by p21-activated kinase modulates intestional smooth muscle contractility. *J Biol Chem*, 288:1200–1213.

Connell, M., Cabernard, C., Ricketson, D., Doe, C., and Prehoda, K. (2011). Asymmetric cortical extension shifts cleavage furrow position in Drosophila neuroblasts. *Mol Biol Cell*, 22:4220–4226.

Dasgupta, U., Bamba, T., Chiantia, S., Karim, P., Tayoun, A. A., Yonamine, I., Rawat, S., Rao, R., Nagashima, K., Fukusaki, E., Puri, V., Dolph, P., Schwille, P., Acharya, J., and Acharya, U. (2009). Ceramide kinase regulates phospholipase C and phosphotidylinositol 4,5,bisphosphate in phototransduction. *Proc Natl Acad Sci*, 106:20063–20068.

D'Avino, P., Takeda, T., Capalbo, L., Zhang, W., Lilley, K., Laue, E., and Glover, D. (2008). Interaction between Anillin and RacGAP50C connects the actomyosin contractile ring with spindle microtubules at the cell division site. *J Cell Sci*, 121:1151–1158.

Dawes-Hoang, R., Parmar, K., Christiansen, A., Phelps, C., Brand, A., and Wieschaus, E. (2005). folded gastrulation, cell shape change, and the control of myosin localization. *Development*, 132:4165–4178.

Dean, S., Rogers, S., Stuurman, N., Vale, R., and Spudich, J. (2005). Distinct pathways control recruitment and maintenance of myosin II at the cleavage furrow during cytokinesis. *Proc Natl Acad Sci*, 102:13473–13478.

Dietzl, G., Chen, D., Schnorrer, F., Su, K., Barinova, Y., Fellner, M., Gasser, B., Kinsey, K., Oppel, S., Scheiblauer, S., Couto, A., Marra, V., Keleman, K., and Dickson, B. (2007). A genome-wide transgenic RNAi library for conditional gene activation in Drosophila. *Nature*, 448:151–156.

Doe, C. (2008). Neural stem cells: balancing self-renewal with differentiation. *Development*, 135:1575–1587.

Doube, M., Klosowski, M., Arganda-Carreras, I., Cordelières, F., Dougherty, R., Jackson, J., Schmid, B., Hutchinson, J., and Shefelbine, S. (2010). BoneJ: free and extensible bone image analysis in ImageJ. *Bone*, 47:1076–1079.

Douglas, M., Davies, T., Joseph, N., and Mishima, M. (2010). Aurora B and 14-3-3 coordinately regulate clustering of centralspindlin during cytokinesis. *Curr Biol*, 20:927–933.

Emery, G., Hutterer, A., Berdnik, D., Mayer, B., Wirtz-Peitz, F., Gaitain, M., and Knoblich, J. (2005). Asymmetric Rab11 endosomes regulate delta cycling and specify cell fate in the Drosophila nervous system. *Cell*, 122:763–773.

Fuse, N., Hisata, K., Katzen, A., and Matsuzaki, F. (2003). Heterotrimeric G proteins regulate daughter cell size asymmetry in Drosophila neuroblast divisions. *Curr Biol*, 13:947–954.

Glotzer, M. (2004). Cleavage furrow positioning. *J Cell Biol*, 164:347–351.

Goeckeler, Z., Masaracchia, R., Zeng, Q., Chew, T., Gallagher, P., and Wysolmerski, R. (2000). Phosphorylation of myosin light chain kinase by p21-activated kinase PAK2. *J Biol Chem*, 275:18366–18374.

Gotta, M. and Ahringer, J. (2001). Distinct role for Gα and G$\beta\gamma$ in regulating spindle position and orientation in Caenorhabditis elegans embryos. *Nature Cell Biol*, 3:297–300.

Green, R., Paluch, E., and Oegema, K. (2012). Cytokinesis in animal cells. *Annu Rev Cell Dev Biol*, 28:29–58.

Gregory, S., Ebrahimi, S., Milverton, J., Jones, W., Bejsovec, A., and Saint, R. (2008). Cell division requires a direct link between microtubule-bound RacGAP and anillin in the contractile ring. *Curr Biol*, 18:25–29.

Grosskortenhaus, R., Pearson, B., Marusich, A., and Doe, C. (2005). The neuroblast gene expression "clock" is regulated by cytokinesis-dependent and cell cycle-independent mechanisms. *Dev Cell*, 8:193–202.

Guan, Z., Prado, A., Melzig, J., Heisenberg, M., Nash, H., and Raabe, T. (2000). Mushroom body defect, a gene involved in the control of neuroblast proliferation in Drosophila, encodes a coiled-coil protein. *Proc Natl Acad Sci USA*, 97:8122–8127.

Hickson, G., Echard, A., and O'Farrell, P. (2006). Rho kinase controls cell shape changes during cytokinesis. *Curr Biol*, 16:359–370.

Izumi, Y., Ohta, N., Hisata, K., Raabe, T., and Matsuzaki, F. (2006). Drosophila Pins-binding protein M regulates spindle-polarity coupling and centrosome organization. *Nat Cell Biol*, 8:586–593.

Joregensen, P. and Tyers, M. (2004). How cells coordinate growth and division. *Curr Biol*, 14:R1014–R1027.

Kaltschmidt, J., Davidson, C., Brown, N., and Brand, A. (2000). Rotation and asymmetry of the mitotic spindle direct asymmetric cell divisions in the developing central nervous system. *Nat Cell Biol*, 2:7–12.

Karpova, N., Bobinnec, Y., Fouix, S., Huitorel, P., and Debec, A. (2006). Jupiter, a new Drosophila protein associated with microtubules. *Cell Motil Cytoskeleton*, 63:301–312.

Keating, H. and White, J. (1998). Centrosome dynamics in early embryos of Caenorhabditis elegans. *J Cell Sci*, 111:3027–3033.

Knoblich, J. (2008). Mechanisms of asymmetric cell division. *Cell*, 132:583–597.

Koh, Y., Popova, E., Thomas, U., Griffith, L., and Budnik, V. (1999). Regulation of DLG localization at synapses by CaMKII-dependent phosphorylation. *Cell*, 98:353–363.

Kondo, T., Hamao, K., Kamijo, K., Kimura, H., Morita, M., Takahashi, M., and Hosoya, H. (2011). Enhancement of myosin II/actin turnover at the contractile ring induces slower furrowing in dividing HeL cells. *Biochem J*, 435:569–576.

Kunda, P., Pelling, A., Liu, T., and Baum, B. (2008). Moesin controls the cortical rigidity, cell rounding, and spindle morphogenesis during mitosis. *Curr Biol*, 18:91–101.

Leberer, E., Dignard, D., Thomas, D., and Leeuw, T. (2000). A conserved Gbeta binding (GBB) sequence motif in Ste20/PAK family protein kinases. *Biol Chem*, 381:427–431.

Lee, C., Robinson, K., and Doe, C. (2006). Lgl, Pins and aPKC regulate neuroblast self-renewal versus differentiation. *Nature*, 439:594–598.

Leeuw, T., Wu, C., Schrag, J., Whiteway, M., Thomas, D., and Leberer, E. (1998). Interaction of a G-protein β-subunit with a conserved sequence in Ste20/PAK family protein kinases. *Nature*, 391:181–185.

Lekomtsev, S., Su, K., Pye, V., Blight, K., Sundaramoorthy, S., Takaki, T., Collinson, L., Cherepanov, P., Divecha, N., and Petronzcki, M. (2012). Centralspindlin links the mitotic spindle to the plasma membrane during cytokinesis. *Nature*, 491:276–279.

Liu, J., Fairn, G., Ceccarelli, D., Sicheri, F., and Wilde, A. (2012). Cleavage furrow organization requires PIP_2-mediated recruitment of anillin. *Curr Biol*, 22:64–69.

Minestrini, G., Harley, A., and Glover, D. (2003). Localization of Pavarotti-KLP in living Drosophila embryos suggests roles in reorganizing the cortical cytoskeleton during the mitotic cycle. *Mol Biol Cell*, 14:4028–4038.

Moutinho-Santos, T., Sampaio, P., Amorim, I., Costa, M., and Sunkel, C. (1999). In vivo localization of the mitotic POLO kinase shows a highly dynamic association with the mitotic apparatus during early embryogenesis in Drosophila. *Biol Cell*, 91:585–596.

Nipper, R., Siller, K., Smith, N., Doe, C., and Prehoda, K. (2007). Gαi generates multiple Pins activation states to link cortical polarity and spindle orientation in Drosophila neuroblasts. *Proc Natl Acad Sci USA*, 104:14306–14311.

O'Connell, C., Wheatley, S., Ahmed, S., and Wang, Y. (1999). The small GTP-binding protein Rho regulates cortical activities in cultured cells during division. *J Cell Biol*, 144:305–313.

Oliferenko, S., Chew, T., and Balasubramanian, M. (2009). Positioning cytokinesis. *Genes Dev*, 23:660–674.

Ou, G., Stuurman, N., D'Ambrosio, M., and Vale, R. (2010). Polarized myosin produces unequal-size daughters during asymmetric cell division. *Science*, 300:677–680.

Piekny, A. and Glotzer, M. (2008). Anillin is a scaffold protein that links rhoA, actin, and myosin during cytokinesis. *Curr Biol*, 18:30–36.

Rolls, M., Albertson, R., Shih, H., Lee, C., and Doe, C. (2003). Drosophila aPKC regulates cell polarity and cell proliferation in neuroblasts and epithelia. *J Cell Biol*, 163:1089–1098.

Rosenblatt, J. (2008). Mitosis: moesin and the importance of being round. *Curr Biol*, 18:91–101.

Rosenblatt, J., Cramer, L., Baum, B., and McGee, K. (2004). Myosin II-dependent cortical movement is required for centrosome separation and positioning during mitotic spindle assembly. *Cell*, 117:361–372.

Royou, A., Field, C., Sisson, J., Sullivan, W., and Karess, R. (2004). Reassessing the role and dynamics of nonmuscle myosin II during furrow formation in Drosophila embryos. *Mol Biol Cell*, 15:838–850.

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

Savoian, M., Goldberg, M., and Reider, C. (2000). The rate of poleward chromosome movement is attenuated in Drosophila zw10 and rod mutants. *Nature Cell BIol*, 2:948–952.

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

Schiel, J. and Prekeris, R. (2010). Making the final cut – mechanisms mediating the abscission step of cytokinesis. *The ScientificWorld J*, 10:1424–1434.

Schwave, T., Bainton, R., Fetter, R., Heberlein, U., and Gaul, U. (2005). GPCR signaling is required for blood-brain barrier formation in Drosophila. *Cell*, 123:133–144.

Shen, L., Weber, C., and Turner, J. (2008). The tight junction protein complex undergoes rapid and continuous molecular remodeling at steady state. *J Cell Biol*, 181:683–695.

Siegrist, S. and Doe, C. (2006). Extrinsic cues orient the cell division axis in Drosophila embryonic neuroblasts. *Development*, 133:529–536.

Siller, K., Cabernard, C., and Doe, C. (2006). The NuMa-related Mud protein binds Pins and regulates spindle orientation in Drosophila neuroblasts. *Nature Cell BIol*, 8:594–600.

Siller, K., Serr, M., Steward, R., Hays, T., and Doe, C. (2005). Live imaging of Drosophila brain neuroblasts reveals a role for Lis1/Dynactin in spindle assembly and mitotic checkpoint control. *Mol Biol Cell*, 16:5127–5140.

Silverman-Gavrilla, R., Hales, K., and Wilde, A. (2008). Anillin-mediated targeting of Peanut to pseudocleavage furrows is regulated by the GTPase Ran. *Mol Biol Cell*, 19:3735–3744.

Straight, A., Field, C., and Mitchison, T. (2005). Anillin binds nonmuscle myosin II and regulates the contractile ring. *Mol Biol Cell*, 26 Pages = 193-201,.

Tse, Y., Piekny, A., and Glotzer, M. (2011). Anillin promotes astral microtubule-directed cortical myosin polarization. *Mol Biol Cell*, 22:3165–3175.

Uehara, R., Goshima, G., Mabuchi, I., Vale, R., Spudich, J., and Griffis, E. (2010). Determinants of myosin II cortical localization during cytokinesis. *Curr Biol*, 20:1080–1085.

von Dassow, G. (2009). Concurrent cues for cytokinetic furrow induction in animal cells. *Trends Cell Biol*, 19:165–173.

Watanabe, S., Ando, Y., Yasuda, S., Hosoya, H., Watanabe, N., Ishizaki, T., and Narumiya, S. (2008). mDia2 induces the actin scaffold for the contractile ring and stabilizes its position during cytokinesis in NIH3T3 cells. *Mol Biol Cell*, 19:2328–38.

Wolfe, B., Takaki, T., Petronczki, M., and Glotzer, M. (2009). Polo-like kinase 1 directs assembly of the HsCyk4 and blocks its recruitment to the central spindle. *PLOS Biol*, 7.

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 Iscuteable apical localization. *Cell*, 100:399–409.

Zhou, Q., Kee, Y., Poiriet, C., Jelinek, C., Osborne, J., Divi, S., Surcel, A., Will, M., Eggert, U., Müller-Taubenberger, A., Iglesias, P., Cotter, R., and Robinson, D. (2010). 14-3-3 coordinates microtubules, Rac, and myosin II to control cell mechanics and cytokinesis. *Curr Biol*, 20:1881–1889.