Patronin/Shot Cortical Foci Assemble the Noncentrosomal Microtubule Array that Specifies the *Drosophila* Anterior-Posterior Axis

**Highlights**

- The *Drosophila* spectraplakin, Shot, recruits Patronin to form noncentrosomal MTOCs
- The actin-binding domain of Shot anchors the ncMTOCs to the oocyte cortex
- Par-1 excludes Shot from the posterior cortex to define the anterior-posterior axis
- Shot/Patronin ncMTOCs lack γ-tubulin and grow MTs from stabilized minus-end stumps

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**In Brief**

Many differentiated cell types lack centrosomes but still form highly polarized microtubule networks. Nashchekin et al. describe how the spectraplakin Shot and the microtubule minus-end-binding protein Patronin form a cortical noncentrosomal microtubule organizing center that acts a source of growing microtubules independently of γ-tubulin.
Patronin/Shot Cortical Foci Assemble the Noncentrosomal Microtubule Array that Specifies the *Drosophila* Anterior-Posterior Axis

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SUMMARY

Noncentrosomal microtubules play an important role in polarizing differentiated cells, but little is known about how these microtubules are organized. Here we identify the spectraplakin, Short stop (Shot), as the cortical anchor for noncentrosomal microtubule organizing centers (ncMTOCs) in the *Drosophila* oocyte. Shot interacts with the cortex through its actin-binding domain and recruits the microtubule minus-end-binding protein, Patronin, to form cortical ncMTOCs. Shot/Patronin foci do not co-localize with γ-tubulin, suggesting that they do not nucleate new microtubules. Instead, they capture and stabilize existing microtubule minus ends, which then template new microtubule growth. Shot/Patronin foci are excluded from the oocyte posterior by the Par-1 polarity kinase to generate the polarized microtubule network that localizes axis determinants. Both proteins also accumulate apically in epithelial cells, where they are required for the formation of apical-basal microtubule arrays. Thus, Shot/Patronin ncMTOCs may provide a general mechanism for organizing noncentrosomal microtubules in differentiated cells.

INTRODUCTION

Many differentiated animal cells and all plant cells lack functional centrosomes, yet form highly organized microtubule (MT) arrays that play essential roles in cell polarity, organization, and function (Bartolini and Gundersen, 2006). For example, both *Drosophila* and rodent hippocampal neurons develop normally without active centrosomes, with the latter extending and even regenerating axons independently of centrosomal MT nucleation (Nguyen et al., 2011; Stiess et al., 2010). Most *Drosophila* tissues lack functional centrosomes or microtubule organizing centers (MTOCs) in interphase (Rogers et al., 2008).

Anterior-posterior axis formation in the *Drosophila* oocyte provides a well-studied example of the role of noncentrosomal MTs. Although the oocyte contains centrosomes, which cluster near the nucleus, oogenesis proceeds normally in their absence (Basto et al., 2006; Januschke et al., 2006; Stevens et al., 2007).

Instead, the majority of MTs grow from the anterior/lateral cortex, but not from the posterior, where the plus ends concentrate (Clark et al., 1994, 1997; Parton et al., 2011; Theurkauf et al., 1992). This noncentrosomal MT array directs the localization of bico (mRNA to the anterior and posterior poles of the oocyte, respectively, to define the main body axis of the embryo (St Johnston, 2005; Zimyanin et al., 2008). 3D modeling of the oocyte MT cytoskeleton has shown that restricting MT minus ends to the anterior/lateral cortex is sufficient to generate an MT network that can direct the transport of oskar mRNA to the oocyte posterior by kinesin (Khuc Trong et al., 2015).

The formation of this polarized MT array is under the control of the PAR proteins, which localize in mutually antagonistic anterior and posterior cortical domains (Doerflinger et al., 2010; Shulman et al., 2000). The posterior crescent of the Par-1 kinase transmits this cortical polarity to the MT cytoskeleton by excluding minus ends from the oocyte posterior. It is not known, however, how PAR-1 activity is transduced into the asymmetric organization of MT minus ends, nor how the minus ends associate with the anterior/lateral cortex.

The recent discovery of the Patronin family of MT minus-end-binding proteins, consisting of Patronin in *Drosophila*, CAMSAP1, 2, and 3 in mammals, and PTHR1 in worms, has begun to reveal how the minus ends of noncentrosomal MTs are organized and maintained (Akhmanova and Steinmetz, 2015; Baines et al., 2009; Goodwin and Vale, 2010; Marcotte et al., 2014; Meng et al., 2008; Richardson et al., 2014). The Patronins recognize and stabilize free MT minus ends by protecting them from depolymerization (Goodwin and Vale, 2010; Hendershot and Vale, 2014; Jiang et al., 2014). Patronins appear to play a particularly important role in organizing MTs in differentiated cells. CAMSAP3 localizes to the apical domain in epithelial cells, where it is required for the formation of the apical-basal microtubule array. CAMSAP2 stabilizes neuronal MTs in axon and dendrites, and its knockdown leads to defects in axon specification and dendritic branch formation (Yau et al., 2014). Similarly, *Caenorhabditis elegans* PTHR1 is required for normal neurite morphology and axon regeneration (Chuang et al., 2014; Marcotte et al., 2014; Richardson et al., 2014). The function of *Drosophila* Patronin has only been examined in cultured S2 cells, where its depletion leads to a decrease in MT number and an increase in free moving MTs (Goodwin and Vale, 2010).

Although it is now clear that the Patronins play an important role in organizing noncentrosomal MTs in differentiated cells, little is known about the regulation of the distribution and activity of...
the Patronins themselves. Here we show that Patronin is recruited to the anterior/lateral cortex of the Drosophila oocyte by the spectraplakin, Shot, under the control of Par-1. These Shot/Patronin complexes form the cortical noncentrosomal MTOCs that organize the polarized MT network in the oocyte, which specifies the anterior-posterior axis.

RESULTS

Shot Is Required for the Polarized Organization of MTs in the Oocyte

We previously isolated 11 new alleles of short stop (shot) in a screen for dominant suppressors of the bicaudal phenotype caused by mislocalizing oskar mRNA to the oocyte anterior (Chang et al., 2011). Shot is the single Drosophila spectraplakin, a giant cytoskeletal linker protein with an N-terminal actin-binding domain and two C-terminal domains that bind MT, the GAS2 domain, which binds to the MT lattice, and a more C-terminal domain that associates with MT plus ends through the +TIP, EB1 (Applewhite et al., 2010; Sun et al., 2001). Null alleles of shot block the specification of the oocyte, and this is also the case for 10 out of 11 of the new alleles (Roper and Brown, 2004). Some germline clones of shot

fail to localize to the oocyte posterior in shot

germline clones (Figures 1A and 1B). To determine whether Shot is specifically required for oskar mRNA localization or plays a more general role in kinesin-dependent transport to the posterior, we also examined the localization of Dynein and the dynactin subunit, Glued, which are transported to the oocyte posterior by kinesin independently of oskar mRNA (Brendza et al., 2002; Palacios and St Johnston, 2002). Neither Dynein nor Glued are localized in shot

ococytes, indicating that either kinesin activity is inhibited or the MT plus ends are not concentrated at the posterior pole (Figure 1C).

We next examined the overall organization of the MTs in fixed and living oocytes. Staining of fixed oocytes with anti-α-tubulin and in vivo labeling of MTs in living oocytes with Jupiter-GFP (Karpova et al., 2006) reveals the anterior-posterior gradient of MTs in wild-type with the highest concentration of MTs at the anterior (Figures 1D and 1E; Movie S1). This anterior enrichment is lost in shot

and the MT organization becomes somewhat variable, with a much more even distribution throughout the oocyte cytoplasm (Figures 1D and 1E, right panels; Movie S2).

Par-1 Regulates the Association of the Shot Actin-Binding Domain with the Cortex

Shot localizes to the anterior and lateral cortex of the oocyte, but is absent from the posterior, following the predicted distribution of MT minus ends. Shot is also strongly enriched at the apical side of the epithelial follicle cells that surround the developing egg chamber (Figure 2A, left). YFP-tagged Shot expressed from a transgenic bacterial artificial chromosome (BAC) rescuing
The construct shows an identical distribution in both the follicle cells and oocyte. We therefore examined whether the interaction of Shot with the oocyte cortex is under the control of the cortical Par proteins that control the polarity of the MT cytoskeleton. In par-1 mutant oocytes, MTs grow from the posterior cortex as well as the anterior/lateral cortex, and the MT cytoskeleton loses its asymmetry, whereas Par-1T786A, which has a uniform cortical distribution, abolishes all MT growth from the cortex (Doerflinger et al., 2010; Parton et al., 2011). Shot responds to Par-1 activity in the same way as MTs: it extends around the posterior in the absence of Par-1, and is lost from the cortex in oocytes over-expressing Par-1T786A (Figures 2A and 2B). Thus, Shot is downstream of Par-1, consistent with it playing a role in MT minus-end localization.

Sequencing of shot reveals that it is a point mutation in the first calponin homology domain of the N-terminal actin-binding domain (ABD) of Shot, changing Val224 to Asp (Figures 2A and 2B, par-1 mutant). Shot also localizes to the apical cortex of the follicle cells. Arrows point to the cortical Shot signal in the oocyte and to the underlying apical signal in the epithelial follicle cells (A, B, D). Arrowheads in (A) point to posterior. Arrows in (E) indicate the cortical signal. Scale bars represent 10 μm.
contacts F-actin (Hanein et al., 1998). In agreement with this, Shot loses its association with the actin-rich cortex in Shot and is mainly cytoplasmic (Figure 2D). Like full-length Shot, the Shot ABD is enriched at the anterior-lateral cortex (Figure 2E, left). Introducing the Val224 to Asp mutation into the Shot ABD disrupts its cortical localization, although the protein still shows an enrichment at the ring canals, which is not observed with the full-length protein (Figure 2E, right). Thus, Shot is recruited to the cortex through its ABD, presumably by direct binding to cortical F-actin, and this interaction is inhibited at the posterior by Par-1.

**Shot Recruits Patronin Foci to the Oocyte Cortex**

We took advantage of the recent identification of Patronin/CAMSAP as an MT minus-end-binding protein to analyze the relationship between cortical Shot and the distribution of MT minus ends in the oocyte (Goodwin and Vale, 2010; Jiang et al., 2014). Live imaging of both transgenic and endogenously tagged Patronin reveals that it localizes to anterior/lateral cortex in the expected distribution of MT minus ends (Figures 3A and 3B; Movie S3, left panel). Importantly, Patronin co-localizes with Shot in distinct cortical foci (Figure 3C). Patronin localization is Shot dependent, as it becomes largely cytoplasmic in shot mutant oocytes (Figure 3D and Movie S3, right panel). Furthermore, the cortical Patronin foci extend around the posterior cortex in par-1 mutant oocytes, as Shot does, consistent with the two proteins being in the same complex (Figure 3E). In agreement with this, Patronin co-immunoprecipitates with Shot-YFP from ovary extracts (Figure 3F). The fact that both Patronin and Shot are no longer cortical in shot indicates that Shot anchors Patronin to the cortex, providing an explanation of how the asymmetric localization of Shot controls the polarized distribution of MT minus ends in the oocyte.

**Patronin Cortical Foci Are Noncentrosomal MTOCs**

It has previously been shown, using Tau-GFP to label MTs and EB1-GFP to label the growing MT plus ends, that oocyte MTs grow out from noncentrosomal foci that can be visualized using an MT regrowth assay (Parton et al., 2011). Upon colcemid treatment, both proteins accumulate in cortical foci. Local inactivation of the colcemid with a pulse of UV light allows MTs to regrow from the cortex (Figure 4A). We therefore examined whether the MTs grow from the Patronin foci. Both EB1-GFP and Tau-GFP accumulate in the cortical Patronin foci upon colcemid treatment, indicating that these contain stable MT minus ends (Figures 4B and S1B). Furthermore, after colcemid inactivation...
with UV light, EB1-GFP and Tau-GFP label growing MTs that emerge from the Patronin foci (Figures 4C, S1C, and S1D; Movies S4 and S5). The Patronin foci also act as a source of growing MTs under steady-state conditions in the absence of colcemid (Figure 4D and Movie S6). After colcemid inactivation, each Patronin focus produces an average of 11.5 new MTs per minute (n = 15; SEM = 0.75), providing a source of MTs that grow in multiple directions (Figure 4E). Moreover, these foci are the only visible source of growing MTs at the oocyte cortex, strongly suggesting that they represent the noncentrosomal, cortical MT organizing centers (ncMTOCs) from which MTs grow to form the polarized cytoskeleton in the oocyte.

In shot2A2 mutant oocytes, many of the foci fail to be retained at the oocyte cortex and redistribute throughout the oocyte cytoplasm, consistent with the loss of most Shot and Patronin from the cortex in this mutant (Figure 4F and Movie S7). These cytoplasmic foci remain active, however, producing growing MTs after colcemid inactivation, explaining why the overall polarity of the MT network is disrupted (Movie S7).

**Patronin Is Required for ncMTOC Formation**

A patronin null mutant blocks oogenesis at an early stage. To test whether Patronin is required for the activity of the cortical ncMTOCs in the oocyte, we therefore used a hypomorphic allele, patronin05252, which strongly reduces Patronin levels (Bellen et al., 2004). patronin05252 homozygous oocytes contain 90% fewer cortical EB1-GFP foci after colcemid treatment than wild-type, and the remaining foci also generally recruit less EB1-GFP (Figures 5A and 5B). Nevertheless, the Patronin foci that form are still active, acting as a source of growing MTs after colcemid inactivation (Figure 5C and Movie S8). The density of MTs is also significantly reduced in patronin05252 clones, as expected from the reduced number of cortical ncMTOCs (Figure 5D). Despite the dramatic reduction in MT number, there are still sufficient MTs to direct the localization of Staufen/oskar mRNA complexes to the oocyte posterior, although the levels of localization are reduced by >40% (Figures 5E and 5F).

**Patronin ncMTOCs Do Not Co-localize with γ-Tubulin**

To further investigate the nature of the Shot/Patronin noncentrosomal MTOCs, we asked whether they contain γ-tubulin as the source of new MTs. Antibody staining of oocytes for γ-tubulin label only the centrosomes adjacent to the oocyte nucleus, but overexpressed γ-tubulin 37C-GFP is also seen in weak foci along the anterior/lateral cortex (Januschke et al., 2006; Parton et al., 2011). We therefore co-expressed γ-tubulin-GFP and Cherry-Patronin to determine whether the two proteins co-localize (Figures 6A and 6A’). Patronin labels some of the nuclear-associated, γ-tubulin foci, which probably correspond to the active centrosomes. The cortical Patronin foci do not co-localize with the γ-tubulin-GFP foci, however, and Shot/Patronin ncMTOCs contain no detectable γ-tubulin. Since MTs start to grow out from Patronin foci within 1 s of colcemid inactivation,
and these foci are the only visible source of cortical MTs, it seems most likely that the MTs are seeded from Patronin-stabilized MT minus-end stumps and not from de novo nucleation by the $\gamma$-tubulin ring complex.

Overexpression of the centriolar duplication factors dSAS6, dSas4, Sak/PLK4, and Ana2/STIL can promote the formation of acentriolar MTOCs in the oocyte (Dzhindzhev et al., 2010; Peel et al., 2007; Stevens et al., 2010). Moreover, expression of membrane-tethered Cep152/Asl and PLK4 is sufficient to induce formation of ectopic acentriolar MTOCs in mouse oocytes (Coelho et al., 2013). To test whether any of these acentriolar MTOC components are involved in the formation of the Shot/ Patronin ncMTOCs, we co-expressed Cherry-Patronin with Asl-GFP (Figure 6B), Ana2-GFP (Figure 6C), dSas6-GFP, dSas4-GFP, and Sak-GFP (data not shown). None of these proteins co-localize with the Patronin foci, however, indicating that they are not components of the ncMTOCs (Figures 6B and 6C, and data not shown).

An alternative mechanism that can contribute to the formation of new MTs is the severing of existing MTs to generate minus ends that act as seeds for new microtubule growth (Baas and Ahmad, 1992; Lindeboom et al., 2013; Roll-Mecak and Vale, 2006). The mammalian Patronin orthologs, CAMSAP2 and CAMSAP3, associate with the microtubule severing protein, Katanin (Jiang et al., 2014). This association is conserved in Drosophila, as a protein trap insertion that labels endogenous Katanin 80 co-localizes with Patronin in the cortical foci in the oocyte and at the apical side of the follicle cells (Lowe et al.,

Figure 5. Patronin Is Required for the Formation of Cortical MTOCs
(A and B) The number of cortical MTOCs marked by EB1-GFP is reduced in patronin$^{05252}$ mutant oocytes. (A) Images of wild-type (WT; left) and patronin$^{05252}$ mutant (right) oocytes expressing nanos>UAS-EB1-GFP after colcemid treatment. The images are projections of the several z sections spanning the oocyte cortex. (B) Quantification of the number of cortical EB1-GFP foci after colcemid treatment in WT and patronin$^{05252}$ oocytes. ***p < 0.0001. Error bars indicate the SEM.
(C) EB1-GFP foci before (left) and after (right) colcemid inactivation in a patronin$^{05252}$ mutant oocyte. Close-up still images from Movie S8. The arrows indicate two of the activated MTOCs.
(D) MT density is strongly reduced in patronin$^{05252}$ mutant oocytes. WT (left) and patronin$^{05252}$ mutant (right) oocytes stained with anti-tubulin.
(E and F) Localization of Stau-GFP to the oocyte posterior is reduced in patronin$^{05252}$ mutant oocytes. (E) Localization of Stau-GFP in WT (left) and patronin$^{05252}$ mutant (right) oocytes. (F) Quantification of the mean fluorescence intensity of posteriorly localized Stau-GFP in patronin$^{05252}$ and WT oocytes. ***p = 0.0005. Error bars indicate the SEM.
Scale bars represent 10 $\mu$m.
leads to a random orientation of MTs (Toya et al., 2016). The mammalian Patronin homolog, CAMSAP3, localizes to the apical cortex of mouse intestinal cells and human Caco2 cells, and mutation of the lian Patronin homolog, CAMSAP3, localizes to the apical cortex (Callao et al., 1989; Jankovics and Brunner, 2006). The mammalian Patronin foci (Figure 7C and Movie S9). Although Jupiter-GFP reveals that most MTs grow from the region of adherens junctions (Figure 7B). Live imaging of EB1-GFP and multi-layering (Figures 7G and S4A). This suggests that Patronin apical foci act as ncMTOCs in epithelial cells and that they are crucial for tissue integrity.

Shot also localizes apically in the follicle cells, and the embryonic salivary gland epithelium, and has been proposed to link acentrosomal MT minus ends to medial actomyosin, although this does not appear to require its ABD (Booth et al., 2014; Roper and Brown, 2003). This suggests that Shot may have a similar role as an anchor of Patronin ncMTOCs in epithelial cells. In agreement with previous studies, we observed that Shot is strongly enriched at the apical side of the follicle cells, where it co-localizes with Patronin (Figures 2A and 7A). In homozygous clones of the ABD mutant, shot02A2, Shot protein at the apical cortex is slightly reduced and the protein is found throughout the cytoplasm, indicating that the ABD contributes to efficient apical recruitment (Figure 7E).

To examine the role of Shot in MT organization, we generated clones of shot0, a null mutation (Lee et al., 2000; Roper and Brown, 2003). Mutant clones lose the pronounced apical enrichment of MTs seen in wild-type cells and have fewer MTs than normal, with the remaining MTs mainly along the lateral cortex (Figures 7D and S4B). shot0 mutant cells contain more MTs than patronin mutant cells, however, and the absence of Shot does not disrupt the apical localization of Patronin (Figure 7F).

It has previously been shown that Patronin functions during spindle elongation in the embryo and in interphase S2 cells to protect MT minus ends from the depolymerizing kinesin, Klp10A (kinesin-13), as simultaneous knockdown of Klp10A and Patronin rescues the MT phenotype of Patronin knockdown alone (Goodwin and Vale, 2010; Wang et al., 2013). To ask whether Patronin also antagonizes KLP10a in epithelial cells, we examined the MT phenotype of klp10a patronin double-mutant clones. Loss of KLP10a partially rescues MT abundance in patronin mutant cells, but does not rescue the apical enrichment of MTs, resulting in an MT phenotype that is similar to that seen in shot0 (Figure 7G). By contrast, klp10a has no effect on MT density or organization in shot0 cells (Figure 7H). Thus, Patronin is required both to position MT minus ends apically and to protect them from depolymerization by Klp10A. Shot is not required for Patronin’s activity in protecting MT minus ends, but the fact that shot and klp10a patronin mutants produce
very similar defects in MT organization suggests that Shot and Patronin act in the same pathway to anchor MTs apically. We also tested whether Patronin functions in the oocyte to protect MT minus ends from depolymerization by Klp10A. However, klp10A patronin double-mutant germline clones show the same reduction in MT density as the patronin single mutant, suggesting that Klp10A plays little role in the germline (Figure S5).

DISCUSSION

The polarized arrangement of the MTs in the Drosophila oocyte depends on the posterior crescent of the Par-1 kinase, which excludes MT minus ends from the posterior cortex (Doerflinger et al., 2010; Parton et al., 2011). Here we show that Par-1 acts by preventing the association of Shot with the posterior actin cortex, thereby restricting the formation of noncentrosomal MTOCs to the anterior and lateral cortex. Computer modeling has shown that this asymmetric localization of MT minus ends is sufficient to explain the weakly polarized MT network that directs the transport of oskar mRNA to the posterior pole (Khuc Trong et al., 2015). Thus, the regulation of the interaction of Shot with the cortex by Par-1 transmits cortical PAR polarity into the polarization of the MT cytoskeleton that localizes the axis determinants (Figure S7).

The mechanism by which Par-1 excludes Shot is unknown. The interaction of Shot with the cortex depends on its N-terminal calponin homology domains, which bind to F-actin (Lee and Kolodziej, 2002; Leung et al., 1999). Thus, Par-1 could phosphorylate Shot to inhibit its binding to the cortex. If this is the case, Par-1 would have to modify the activity or accessibility of the N-terminal ABD of Shot, as this domain recapitulates the posterior exclusion and cortical recruitment of the full-length protein. We have not detected any phosphorylation of the ABD by Par-1 in vitro, however, and it seems more likely that Par-1 acts by modifying the cortex to block the binding of Shot.

Shot and its vertebrate ortholog, MACF1, have previously been shown to interact with the MT plus-end tracking protein EB1 through their C-terminal SxIP motifs and with the MT lattice by preventing the association of Shot with the posterior actin cortex, thereby restricting the formation of noncentrosomal MTs. Upon centrosome inactivation in post-mitotic Drosophila trochal cells and C. elegans intestinal cells, γ-TuRC complexes and other pericentriolar material (PCM) components are released from the centrosome and transported toward the apical membrane, where they nucleate MT (Brodi et al., 2010; Feldman and Priess, 2012). Whole MTs released from the centrosome can also be delivered and anchored to the apical domain or cell junctions by Ninein (Lechner and Fuchs, 2007; Mogensen et al., 2000). Alternatively, new MTs can grow from MT ends generated by severing enzymes, a mechanism that is thought to be important in plant cells and neurons (Baas and Ahmad, 1992; Lindboom et al., 2013; Roll-Mecak and Vale, 2006). Here, we present evidence that this latter mechanism is responsible for the formation of the MT array that directs Drosophila axis formation. Firstly, Shot/Patronin ncMTOCs contain stable minus ends even after treatment with the MT-depolymerizing drug, colcemid, as shown by the persistent recruitment of Tau-GFP and EB1-GFP to these foci. This is consistent with the ability of Patronin and CAMPSAPs to capture and stabilize minus ends of single MTs in vitro and in cells (Goodwin and Vale, 2010; Hendershot and Vale, 2014; Jiang et al., 2014; Meng et al., 2008). Secondly, MTs start to grow out in all directions from the Shot/Patronin foci immediately after colcemid inactivation. Indeed all visible growing MTs emanate from Patronin foci.
indicating that they are the principal source of MTs in the oocyte. Thirdly, the foci contain no detectable γ-tubulin and do not co-localize with POM proteins. This is consistent with observations in Caco-2 cells, which showed that CAMSAP2 and CAMSAP3 do not co-localize with γ-tubulin and in the C. elegans epidermis, where PTRN-1 and γ-tubulin function in parallel pathways to assemble circumferential MTs (Tanaka et al., 2012; Wang et al., 2015).

Taken together, these results suggest a model in which the Shot/Patronin foci act as nMTOCs by capturing and stabilizing MT minus-end stumps that then act as templates for new MT growth. One attractive feature of this model is that it uncouples MT organization from MT nucleation in both space and time. The Shot/Patronin complex bypasses the need to continually grow. The number of MTs can even increase in the absence of new MT nucleation if MT-severing proteins chop up existing MTs to produce new minus ends that can then be captured and stabilized. The presence of the severing protein, Katanin, in the Shot/Patronin foci is intriguing in this context, as it raises the possibility that it severs existing MTs to provide a robust anchor to retain MTs at the apical cortex. The combination of Patronin binding to the minus-end stump and Shot binding to the MT lattice may therefore provide a local source of minus ends for Patronin to capture.

Shot and Patronin also co-localize at the apical cortex of the epithelial follicle cells, where they are required for apical-basal MT organization. This consistent with the recent observation that CAMSAP3 is required for the recruitment of MT minus ends to the apical cortex of mammalian intestinal epithelial cells (Toya et al., 2016). Thus, this function of Patronin has been evolutionarily conserved. Furthermore, the similarities between roles of Shot and Patronin in the oocyte and the follicle cells suggest that the complex may provide a general mechanism for organizing non-centrosomal MTs. The relationship between Shot and Patronin is different in the follicle cells compared with the oocyte, however, as Shot is not required for the apical recruitment of Patronin. Nevertheless, loss of either protein produces a very similar loss of apical MT and a reduction in overall MT density. Although we cannot rule out the possibility that they act in parallel pathways, this observation suggests that they collaborate to anchor MTs to the apical cortex. The combination of Patronin binding to the MT minus ends and Shot binding to the MT lattice may therefore provide a robust anchor to retain MTs at the apical cortex.

**EXPERIMENTAL PROCEDURES**

**Colcemid Treatment**

The protocol was modified from Parton et al. (2011). Flies were starved for 3 hr and then fed colcemid (Sigma) in yeast paste (66 g/ml) for 2–3 hr. Ovaries were dissected and imaged as described below. Colcemid was inactivated with a brief UV pulse (3–5 s).

**Imaging**

For live imaging, ovaries were dissected and imaged in Voltaef oil 10S (VWR International) on an Olympus IX81 inverted microscope with a Yokogawa CSU22 spinning disc confocal imaging system (40× 1.35 NA Oil UPlanSapo, 60× 1.35 NA Oil UPlanSapo, and 100× 1.3 NA Oil UPlanSapo). Fixed preparations were imaged using Olympus IX81 (40× 1.35 NA Oil UPlanSapo, 60× 1.35 NA Oil UPlanSapo) and Zeiss LSM510 (40× NA 1.3 Oil Plan-Neofluor) confocal microscopes. Images were collected with Olympus Fluoview, LSM510 AIM software, or MetaMorph software and processed using ImageJ. The oocyte cortex was imaged by collecting 10–15 z sections spaced 0.5 μm apart and then merging them.

**Immunohistochemistry**

Ovaries were fixed for 10 min in 10% paraformaldehyde and 2% Tween in PBS. Ovaries were then blocked with 10% BSA in PBS for 1 hr at room temperature. Ovaries were incubated with the primary antibody for 16 hr in PBS with 0.2% Tween and for 4 hr with the secondary antibody. In situ hybridizations were performed as previously described (Doezinger et al., 2013). We used the following primary antibodies: mouse anti-α-tubulin fluorescein isothiocyanate at 1:250 (Sigma); mouse anti-Dynein heavy chain at 1:50 (DSHB); rabbit anti-Glued antibody raised against amino acid residues 1–400 of Glued and used at 1:100; mouse anti-DIG Cy3 at 1:120 (Jackson Immunoresearch); rabbit anti-Patronin (Goodwin and Vale, 2010) at 1:300 (gift from R. Vale, HHMI and UCSF, USA); mouse anti-Armadillo at 1:100 (DSHB); and guinea pig anti-Shot antibody raised against amino acid residues 2,602–3,640 (isoform PE) and used at 1:500. Conjugated secondary antibodies (Jackson Immunoresearch) were used at 1:100.

**Molecular Biology**

To generate a rescuing genomic shot transgene with C-terminal YFP tag, we used the PACMAN CH031–44M3.BAC clone (fenken et al., 2009) covering the entire shot locus. The BAC was modified using the gAlk positive/counter-selection cassette and recombineering (Warming et al., 2009). Transgenic flies were created by Genetvision.

The Patronin C-terminal YFP knockin was made by injecting nos-Cas9 embryos (Port et al., 2014) with a single guide RNA targeting the region of the stop codon in patronin (5′-GCGGCTTGTAATCTAGAAGGG-3′, the stop codon is in bold) and a donor plasmid with 4-kb homology arms surrounding the Venus sequence.

pUASP-mKate-ABD was constructed by amplifying Shot ABD and mKate2 with the following primers: 5′-ATGTAGCGGCCGCCCCCTAGCCATCCAAGAAGA-3′ and 5′-ATGTATCTTAGATCAAATGACGCTGCTTGTACAGCTCGTC-3′. The amplified fragments were cloned into the pUASP vector (Rorth, 1998). The mutated Shot ABD was amplified with 5′-GATACACTGCCAAGAATCAG-3′ and 5′-GCTATGTTGCCAGTTTGCAGTC-3′. Shot RE cDNA was obtained from A. Prokop (University of Manchester, UK).

For generation of pUASP-­mCherry-­Patronin and pUASP-­mCherry-­Patronin, Patronin RI and mCherry were amplified with 5′-ATGTAGCTTCCACGAGCAAGGGCGGAGAACAA-3′ and 5′-GACATTCCATTAGATAGTTTGCCACG-3′. The amplified fragments were cloned into the pUASP vector (Rorth, 1998). The mutated Patronin RI and mCherry were amplified with 5′-GCAGAATCTGCAAGATGAGAGGAGGAAAGGAAGG-3′ and 5′-GCTATGTTGCCAGTTTGCAGTC-3′. The amplified fragments were cloned into the pUASP vector (Rorth, 1998) and the pUASP vector (Irons et al., 2006).

For generation of pUASP-­mCherry-­Patronin, Patronin RI and mCherry were amplified with 5′-ATGGACAGACTCTGCAAGAGCGCCTGACATGAGG-3′ and 5′-GCGGTATCTAATCCACTAATTAAAACGAGAGGAGGAAAGG-3′. The amplified fragments were cloned into the pUASP vector (Irons et al., 2006).

shot−/− genomic DNA was isolated from homzygous embryos and larvae using the Gentra Puregene Cell Kit (Qiagen), and exonic regions were amplified by PCR and sequenced. Primer sequences are available on request.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures, five figures, and nine movies and can be found with this article online at http://dx.doi.org/10.1016/j.devcel.2016.06.010.

**AUTHOR CONTRIBUTIONS**

D.N. performed most of the experiments and data analysis. A.R.F. performed immunoprecipitations. D.N. and D.St J. planned the experiments. D.N. and D.St J. conceived the project and wrote the manuscript.
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