REPORT

DEVELOPMENTAL BIOLOGY

Symmetry breaking in the female germline cyst

D. Nashchekin[1]*, L. Busby[1], M. Jakobs[2], I. Squires[1]†, D. St. Johnston[1]*

In mammals and flies, only one cell in a multicellular female germline cyst becomes an oocyte, but how symmetry is broken to select the oocyte is unknown. Here, we show that the microtubule (MT) minus end-stabilizing protein Patronin/CAMSAP marks the future *Drosophila* oocyte and is required for oocyte specification. The spectraplakin Shot recruits Patronin to the fusome, a branched structure extending into all cyst cells. Patronin stabilizes more MTs in the cell with the most fusome material. Our data suggest that this weak asymmetry is amplified by Dynein-dependent transport of Patronin-stabilized MTs. This forms a polarized MT network, along which Dynein transports oocyte determinants into the presumptive oocyte. Thus, Patronin amplifies a weak fusome anisotropy to break symmetry and select one cell to become the oocyte.

In many organisms, not all female germ cells develop into oocytes. Some cells become accessory cells that contribute material to the oocyte (*1*). Mouse female germ cells form cysts of up to 30 cells, but most cells undergo apoptosis after transferring cytoplasm and centrosomes to the small number of cells that become oocytes (*2*, *3*). In *Drosophila*, germline cyst formation starts in the germarium, which has three regions. A stem cell produces a cystoblast, which then divides four times with incomplete cytokinesis to generate a cyst of 16 germ cells connected by intercellular bridges, or "ring canals" (*4*, *5*). As the cyst moves through regions 2a and 2b of the germarium, it is surrounded by epithelial follicle cells and then rounds up in region 3 to form a follicle. By this stage, one cell has been selected as the oocyte and the others become nurse cells (Fig. 1A). Oocyte selection depends on the formation of a noncentrosomal microtubule organizing center (ncMTOC) in the future oocyte that organizes a polarized microtubule (MT) network that directs the dynein-dependent transport of cell fate determinants and centrosomes into the pro-oocyte (*6*–*8*) (Fig. 1A). How symmetry is broken to specify which cell contains the ncMTOC and becomes the oocyte is unclear.

Patronin and its vertebrate orthologs (CAMSAPs) are MT minus end-binding proteins that have been recently found to be essential components of ncMTOCs (*9*–*13*). To investigate the role of Patronin in oocyte determination, we examined the distribution of oocyte markers in *patronin*$^{c9\text{-}c5}$ mutant (MUT) cysts (Fig. 1, B and C, and fig. S1). In wild-type (WT) cysts, Orb and centrosomes accumulate in future oocytes in regions 2b to 3 (*14*–*16*) but are rarely localized in *patronin* mutants (24 and 3% of MUT cysts, respectively) (Fig. 1, B and C). Several germ cells enter meiosis in region 2a and accumulate the synaptonemal complex protein C(3)G, which becomes restricted to two cells in region 2b and to the oocyte in region 3 (*17*) (fig. S1). C(3)G is not localized in region 3 of *patronin* cysts, and 44% of the cysts in region 2b have three cells in meiosis (fig. S1). Thus, Patronin is required for oocyte determination.

To determine whether Patronin is asymmetrically distributed in the cyst, we imaged germaria expressing endogenously tagged Patronin-Kate. Patronin starts to accumulate in a single cell in each cyst in region 2a, earlier than other markers for the presumptive oocyte, and remains in one cell in regions 2b to 3, where it forms distinct foci in the cytoplasm (Fig. 2, A and A′). This cell will become the oocyte, because it is also labeled by Orb (Fig. 2B) and C(3)G (Fig. 2C). *patronin* mRNA is not localized within the cyst, and Patronin expressed from a cDNA with heterologous untranslated regions and promoter shows a similar distribution to the endogenous protein, indicating that Patronin is localized as a protein and not by transcription in this cell or by mRNA localization (Fig. 2, B and C, and fig. S2).

Dynein does not localize to the presumptive oocyte in *patronin* MUT cysts (Fig. 3A). This suggests that the loss of Patronin disrupts the formation of the MTOC in the pro-oocyte, leading to the loss of the polarized MT network along which Dynein transports cargoes into one cell. Because most MT plus ends accumulate at the site of MT nucleation, we used the MT plus end-tracking protein EB1-GFP to visualize the putative MTOC in the cyst. Most EB1-GFP comets localize to one cell in regions 2b to 3 (Fig. 3, B and C, and movies S1 and S2). Moreover, the densest EB1-GFP signal colocalizes with the Patronin foci in the same cell, suggesting that the latter are the MTOCs formed in the pro-oocyte (Fig. 3D). This asymmetric distribution of EB1-GFP is lost in *patronin* cysts, where EB1-GFP comets are distributed more homogeneously (Fig. 3, B and C, and movies S3 and S4). Patronin is therefore required for MTOC formation in the presumptive oocyte and for the organization of a polarized MT network.

WT cysts contain a population of stable, acetylated MTs that form along the fusome, an endoplasmic reticulum-, spectrin-, and actin-rich structure that connects all cells of the cyst (*16*–*19*) (fig. S3). In *patronin* MUT cysts, there is a 2.5-fold reduction in stable MTs (Fig. 3E and fig. S3). Thus, in the absence of Patronin, the whole organization of MTs in the cyst is disrupted. Patronin binds MT minus ends and stabilizes MTs by protecting their minus ends against kinesin-13–induced depolymerization (*11*, *13*). Our results suggest that early accumulation of Patronin in only one cell of the cyst stabilizes MT minus ends there, leading to dynein-dependent transport into this cell, the formation of MTOCs, and the subsequent specification of the oocyte.

To determine whether centrosomes contribute to the formation of Patronin MTOCs, we imaged cysts expressing endogenously tagged Patronin-YFP and the centrosomal protein Asterless-Cherry. Although centrosomal clusters localize near Patronin foci, the Asterless and Patronin signals only partially overlap, and most Patronin foci lie outside of the centrosomal cluster, indicating that Patronin MTOCs are noncentrosomal (fig. S4A). Centrosomes have been proposed to be inactive during their migration into the oocyte and they lack crucial components of the pericentriolar material (*8*). To test whether centrosomes contribute to MT organization, we imaged cysts expressing EB1-GFP and Asterless-Cherry. The centrosomes showed strong MT nucleating activity in region 1, where they organize the mitotic spindles (fig. S4B and movie S5). However, only some Asterless-Cherry labeled centrosomes in the presumptive oocyte produce EB1-GFP comets in region 2b (fig. S4C and movie S6). Thus, Patronin-dependent ncMTOCs create the initial asymmetry in MT organization that leads to the accumulation of centrosomes in the pro-oocyte, which may then be amplified by activation of some centrosomes in this cell. The close proximity of the active centrosomes to the ncMTOCs raises the possibility that new MTs produced by these centrosomes are released and then captured and stabilized by Patronin in ncMTOCs, a mechanism described for CAMSAP proteins (*20*).

The observation that Patronin is the earliest known marker for the future oocyte raises the question of how symmetry is broken in the cyst to enrich Patronin in one cell. One proposed mechanism for symmetry breaking is that the cell that inherits the most fusome

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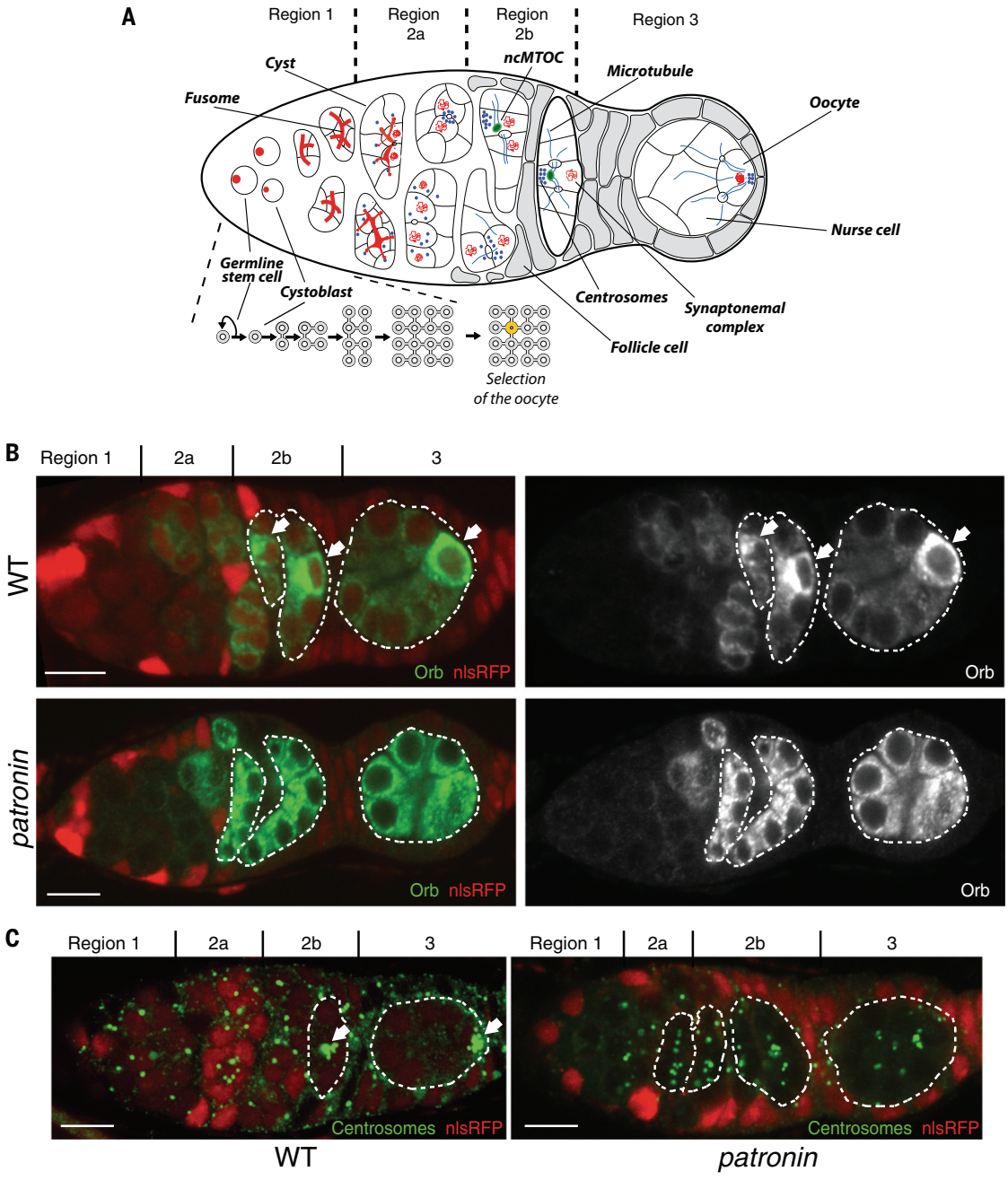

Fig. 1. Patronin is required for oocyte specification. (**A**) Schematic diagram of a *Drosophila* germarium showing germline cyst formation and oocyte selection. (**B** and **C**) Distribution of the oocyte specification markers Orb (B) and centrosomes (C) in WT [top or left in (C)] and *patronin* MUT [bottom or right in (C)] cysts. For all figures, arrows point to the future oocyte, cysts are marked by dashed lines, MUT cysts are labeled by the absence of nuclear localization signal RFP (nlsRFP), and regions of the germarium are indicated on the top. Scale bars, 10 μm.

becomes the presumptive oocyte (*21*). The fusome is asymmetrically partitioned during mitoses in region 1, so that mother cells inherit more material than their daughters, and one of the two cells with four ring canals has more fusome material than the rest (*19*). To determine whether Patronin associates with the fusome, we imaged germaria expressing endogenously tagged Patronin-YFP and the fusome marker Hts-Cherry. Patronin localizes on the fusome in early region 2a but becomes concentrated in one cell as the cyst progresses toward region 3 (Fig. 4A and fig. S5A). When the MTs are depolymerized with colcemid, however, Patronin remains on the fusome in regions 2b and 3 (Fig. 4B). Thus, the fusome determines the initial localization of Patronin in early region 2a, including its slight enrichment in the pro-oocyte, which is then amplified by an MT-dependent process.

The spectraplakin Shot localizes to the fusome, is required for the oocyte specification, and recruits Patronin to ncMTOCs in the oocyte later in oogenesis, making it a good candidate for a factor that links Patronin to the fusome (*13*, *17*). In *shot*$^{-}$ cysts, Patronin does not accumulate in one cell and fails to form foci (Fig. 4C). Furthermore, loss of Shot prevents Patronin from associating with the fusome (Fig. 4C and fig. S5, B and C). Thus,

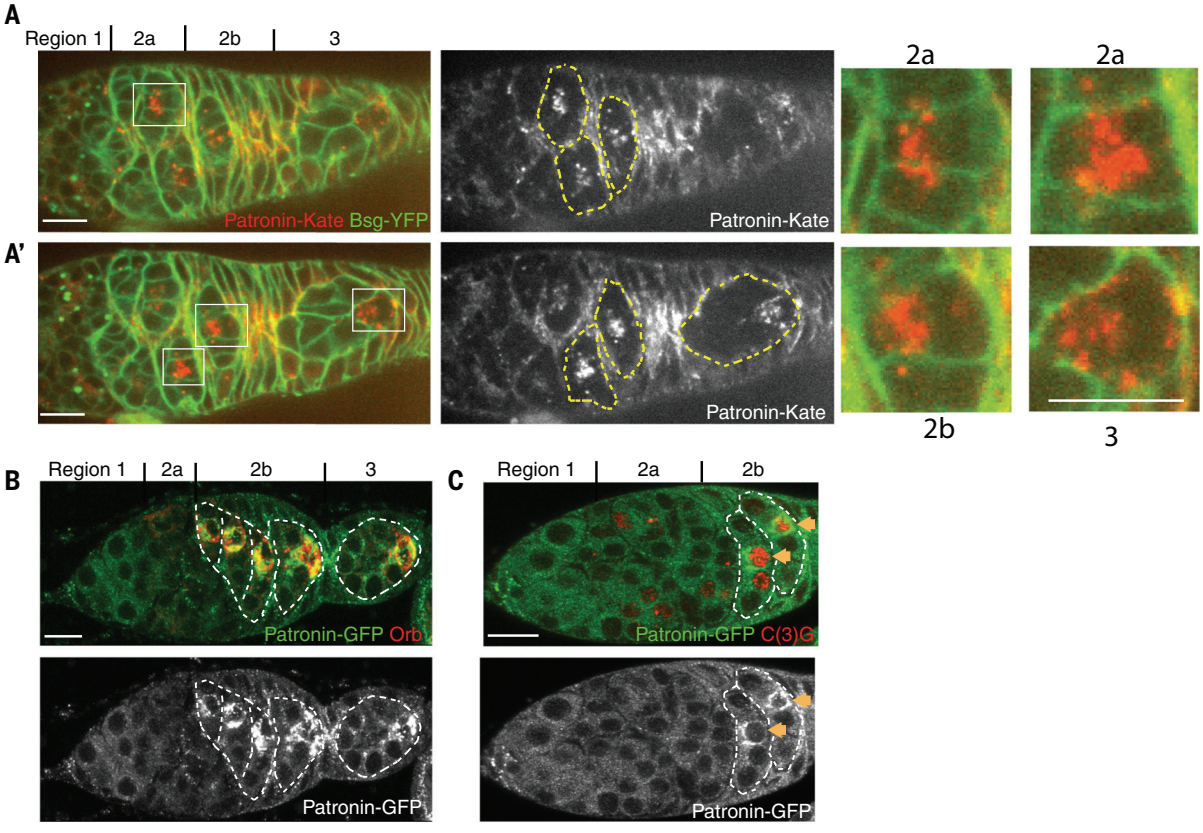

Fig. 2. Patronin accumulates in the future oocyte. (**A** and **A′**) Two different focal planes of a live germarium showing accumulation of endogenously tagged Patronin-Kate in one cell of the cyst. Regions 2a and 2b are shown as close-ups. Cell membranes are labeled by Basigin-YFP (Bsg-YFP). (**B** and **C**) Ectopically expressed ubq>Patronin-GFP accumulates in future oocytes labeled by Orb (B) or C(3)G (C).

Shot is required to recruit Patronin to the fusome, thereby transmitting fusome asymmetry to Patronin localization.

The MT-dependent enrichment of Patronin in one cell as the cyst moves through the germarium suggests that its initial, weakly asymmetric distribution on the fusome is then amplified by Dynein-dependent transport toward the minus ends of the MT that have been stabilized by Patronin. We tested Dynein function by examining components of the Dynein-dynactin complex that are required for oocyte specification: *egl*, *BicD*, and *Arp1* (*22*–*24*), (Fig. 4D and fig. S6, A and B). Like MT depolymerization, mutations in any of these genes disrupt the enrichment of Patronin foci in one cell. Deletion of the MT minus end-binding domain of Patronin, but not the CKK domain (*25*), also prevents Patronin accumulation in the pro-oocyte (fig. S6, C and D). Thus, Patronin localization depends on its binding to MT minus ends and on Dynein activity, suggesting that Dynein transports Patronin bound to MT minus ends toward the pro-oocyte.

Our observations led us to propose a four-step model of cyst polarization and oocyte selection (Fig. 4E). First, during cyst formation, the asymmetric segregation of the fusome leads to the one cell with more fusome material than the rest. Second, in region 2a, Patronin is recruited to the fusome by Shot. The cell with the most fusome material therefore contains more Patronin, leading to the stabilization of more MT minus ends in this cell and a weakly polarized MT network. Third, Patronin-bound MTs in other cells of the cyst are then transported by Dynein along these MTs toward their minus ends in the pro-oocyte. Fourth, this creates a positive feedback loop: As Dynein transports more Patronin and MTs into the cell with the most stabilized MT minus ends, more minus ends become stabilized in this cell, amplifying the MT polarity and leading to enhanced Dynein transport of oocyte determinants into this cell. In this way, the small original asymmetry in the fusome is converted into the highly polarized MT network that concentrates the oocyte determinants in one cell.

Patronin is a member of the conserved CAMSAP family, raising the possibility that the molecular mechanisms of oocyte selection in *Drosophila* could be conserved during the formation of mammalian oocytes. Although fusomes have not been observed in mammalian cysts (*26*), MT-dependent transport of organelles through intercellular bridges has been shown to play an important role in oocyte differentiation in mice (*3*).

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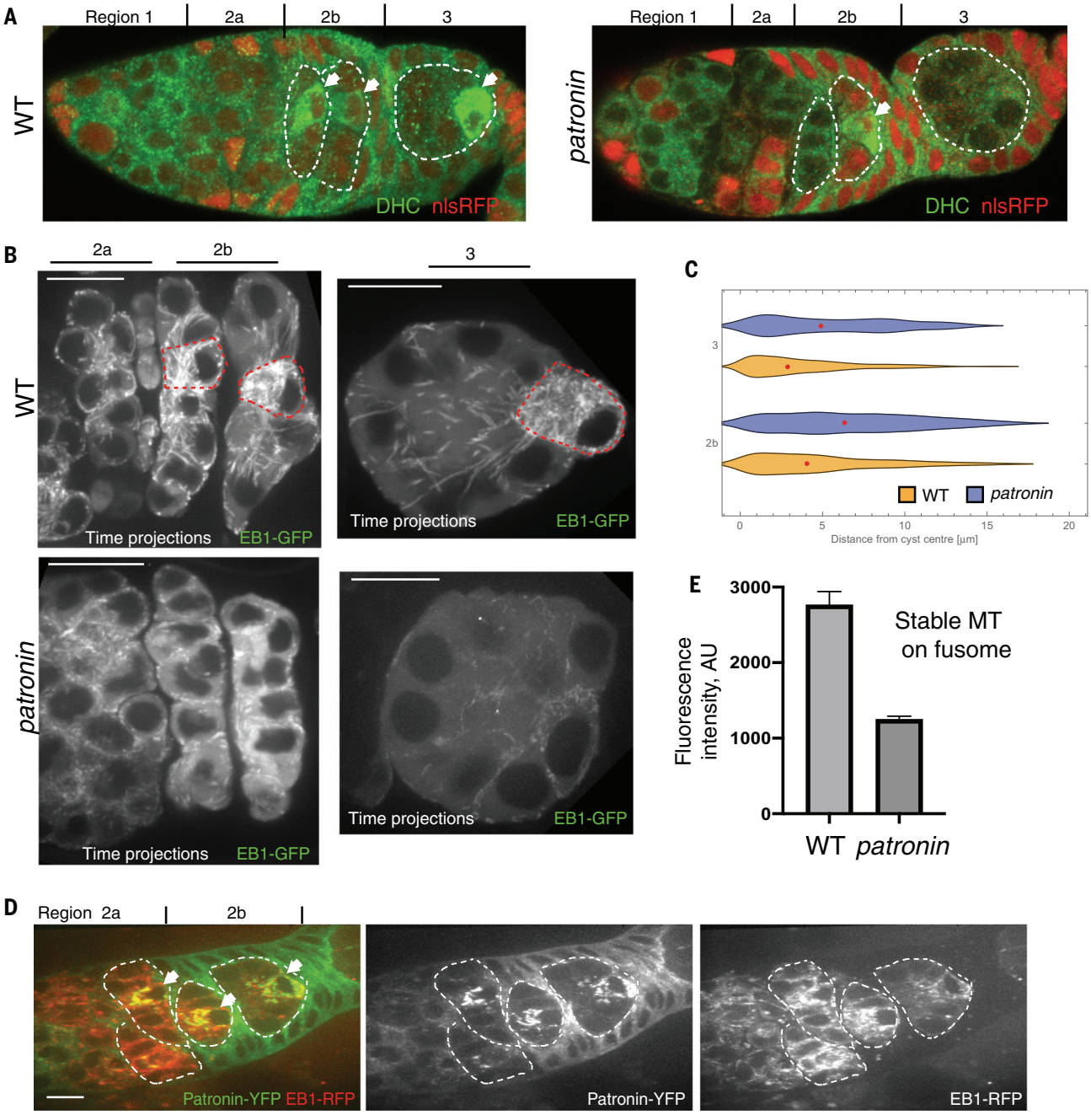

Fig. 3. Patronin is required for MT organization in the cyst. (**A**) Distribution of Dynein heavy chain (DHC) in WT and *patronin* MUT cysts. (**B** to **D**) Patronin is required for MTOC formation in the presumptive oocyte. (B) EB-1 comet tracks in WT (top) and *patronin* MUT (bottom) cysts. The images are projections of several time points from movie S1 (WT; region 2), movie S2 (WT; region 3), movie S3 (*patronin*; region 2), and movie S4 (*patronin*; region 3). The red dashed line marks cells with MTOCs. (C) Quantification of EB-1 comet distribution in WT and *patronin* MUT cysts in region 3 and 2b of the germarium. Red dots indicate median values. (D) Live germarium showing colocalization of Patronin-YFP foci with the MTs plus end marker EB1-GFP in the presumptive oocyte. (**E**) Quantification of the mean fluorescence intensities of fusome-associated acetylated MTs in *patronin* MUT and WT cysts. Errors bars indicate the SEM.

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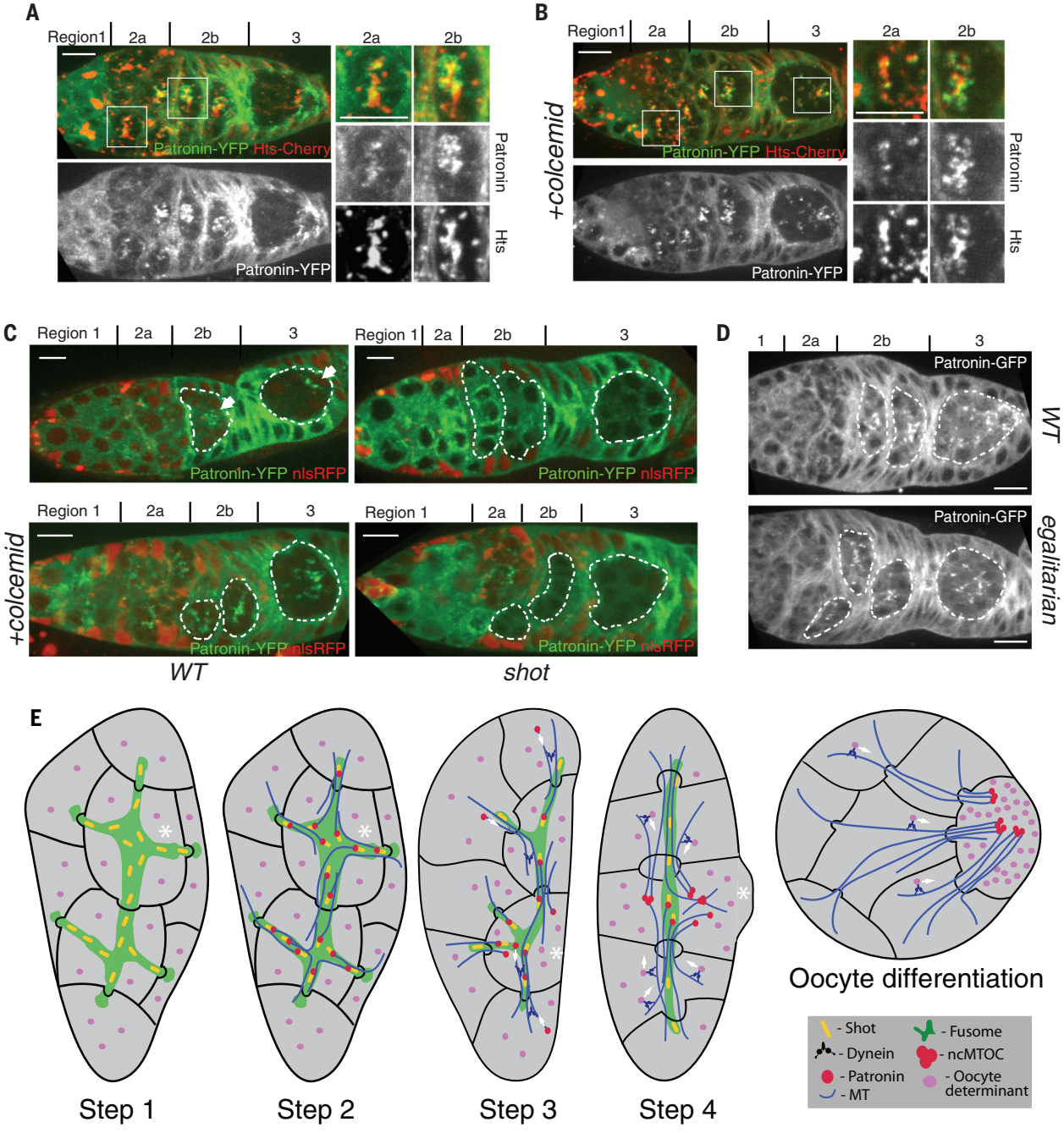

Fig. 4. Patronin localization is defined by the fusome and by a positive feedback loop of Dynein-mediated transport. (**A** and **B**) Patronin associates with the fusome in an MT-dependent manner. Shown are untreated (A) or colcemid-treated (B) live germaria expressing Patronin-YFP and Hts-Cherry. Regions 2a and 2b are shown as close-ups. (**C**) Shot links Patronin to the fusome. Shown are live germaria containing WT (left) and *shot* MUT (right) cysts expressing Patronin-YFP either untreated (top) or treated with colcemid (bottom). (**D**) Patronin localization depends on Dynein activity. Shown are WT (top) and *egalitarian* MUT (bottom) live germaria expressing transgenic Patronin-GFP. (**E**) Diagram showing the four steps in cyst polarization that lead to the specification of the oocyte and its subsequent positioning at the posterior of the cyst in region 3 (see text for details). Asterisk indicates the presumptive oocyte.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
Figs. S1 to S6
References (*27*–*37*)
Movies S1 to S6
MDAR Reproducibility Checklist

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Specifying one oocyte from many

In many animals, only one cell from a cyst of germ cells is selected to become the oocyte. Using fruit flies as a model, Nashchekin *et al*. identified the microtubule minus end-binding protein Patronin/CAMSAP as a key factor for specifying oocyte fate. Patronin amplifies an initial asymmetry provided by the fusome to form a noncentrosomal microtubule network focused on one cell, along which dynein transports oocyte fate determinants. This mechanism for selecting a single oocyte may be shared in other organisms. —BAP

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