Supplemental Videos

Video 1. Symmetric accumulation of *nosGFP during PB formation in a WT embryo (Related to Fig. 1B)**
High resolution confocal time-lapse movie of a 1-2 hr WT embryo expressing *nos**GFP. Posterior is down. Images were captured at 3 frames/s, and each second represents 10 s of elapsed time.

Video 2. Asymmetric accumulation of *nosGFP during PB formation in a *gcl* embryo (Related to Fig. 1B)**
High resolution confocal time-lapse movie of a 1-2 hr *gcl* embryo expressing *nos**GFP. Posterior is down. Images were captured at 3 frames/s, and each second represents 10s of elapsed time.

Video 3. Accumulation of *nosGFP around PB nuclei (Related to Figs. 1D and 1E)**
High resolution confocal time-lapse movie of 1-2 hr WT and *gcl* embryos expressing *nos**GFP. Posterior is to the right. Images were captured at 3 frames/s, and each second represents 10 s of elapsed time. N marks PB nuclei.

Supplemental Experimental Procedures

Imaging

Embryos were prepared for live imaging as described in (Lerit and Gavis, 2011). Briefly, dechorionated embryos were adhered to a sticky 22x30 mm #1.5 glass coverslip and covered with a thin layer of halocarbon oil. Imaging of *nos**GFP was captured at 300 ms intervals and performed on a Leica TCS SP5 confocal microscope with a 100x, 1.49 NA oil immersion objective. Live images of *nos**GFP were post-processed with a 3x3 median filter and normalized for photobleaching using LAS AF (Leica) and ImageJ (NIH) software. Imaging of Asl-YFP and αTub-GFP was collected at 0.5 µm intervals over a 15-20 µm volume at 60 s time intervals and performed on a Nikon Eclipse Ti microscope with 40x, 1.3 NA or 100x, 1.49 NA oil immersion objectives using a spinning disk confocal head (CSU-22, Yokagawa) and an EMCCD camera (Clara; Andor).

Immunofluorescence

Embryos were fixed in a 1:4 solution of 4% paraformaldehyde:heptane for 20 min and devitellinized in methanol. For visualization of MTs, embryos were fixed according to the method of Theurkauf (Theurkauf, 1994). Briefly, embryos were fixed in 1:1 37% paraformaldehyde:heptane for 3 min, rinsed in PBS, and manually devitellinized using 30G PrecisionGlide Needles (BD). For all experiments, embryos were blocked in BBT (PBS supplemented with 0.1% Tween-20 and 0.1% BSA), incubated overnight at 4 ^{o}C with primary antibody in BBT, further blocked in BBT supplemented with 2% normal goat serum, and incubated for 2 hours at room temperature with secondary antibody with DAPI. Embryos were mounted in Aqua-Poly/Mount (Polysciences, Inc.).

The following primary antibodies were used: rabbit anti-Vasa (1:10,000, gift from Ruth Lehmann), rabbit anti-Vasa (1:2,000, gift from Paul Lasko), rabbit anti-Cnn (1:3,000, gift from Tim Megraw), guinea pig anti-Asl (1:3000, gift from Greg Rogers), rabbit anti-Anillin (1:1000, gift from Christine Field), mouse anti-γ-Tubulin ascites GTU88 (1:50, Sigma-Aldrich), mouse anti-α-Tubulin DM1α (1:500, Sigma-Aldrich), rabbit anti-phospho-Histone H3 Ser10 (pH3; 1:1000, Millipore), mouse anti-phospho-Tyrosine 4G10 (1:1000, Millipore). Secondary antibodies: Alexa Fluor 488, 568, or 627 (1:500, Molecular Probes), and DAPI (4',6-diamidino-2-phenylindole, dihydrochloride; 10 ng/mL, ThermoFisher).

Supplemental References

THEURKAUF, W. E. 1994. Immunofluorescence analysis of the cytoskeleton during oogenesis and early embryogenesis. *Methods Cell Biol.,* 44**,** 489-505.