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Supplemental Data

Polar Body Emission Requires a RhoA Contractile Ring and Cdc42-Mediated Membrane Protrusion

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Supplemental Experimental Procedures

Materials

Methyubiquitin was purchased from Boston Biotech (Boston MA) and was dissolved in water to 10 mg/ml. Rhodamine tubulin (Cytoskeleton Inc) was divided in single use aliquots and stored at -70 ^{o}C. Each aliquot was diluted using the buffer provided by the supplier to 2 mg per ml and between 10-20 nl was injected per oocyte. Alexa 594 G-actin (Molecular Probes) was stored at -70 ^{o}C in single use aliquots and diluted using the buffer provided by the supplier to 0.5 mg per ml (10 nl per oocyte). Alexa 594 phalloidin (Molecular Probes) was dissolved in methanol (200 U per ml) and stored at -70 ^{o}C. Prior to injection, the required solution was diluted 5X with water and 30 nl was injected per oocyte. Rabbit polyclonal antibodies against Aurora B were raised against a GST fusion protein containing the N-terminal 128 amino acids of Xenopus Aurora B (Bolton et al., 2002). Affinity purification was carried out by absorbing the IgG fractions to the purified antigen (GST-Aur $B_{1\text{-}128}$) immobilized on Sepharose beads. Affinity purified antibodies against phosphor-myosin light chain (p-MLC) were purchased from New England BioLabs (3671, custom-order). Antibodies against cyclin B were provided by Dr. James Maller. The N3- β5-tubulin-GFP construct (Verlhac et al., 2000) was a gift from Dr. Marie-Helene Verlhac.

Conjugation of Antibodies to Alexa Fluorophores

All antibodies to be conjugated were dialyzed against phosphate buffer saline (PBS, 10 mM sodium phosphate buffer, pH 7.5, 150 mM NaCl) and concentrated to 1 mg per ml. We carried out Alexa fluorephore-antibody conjugation strictly according to the procedure provided by Molecular Probes, using 50-100 μg of antibodies in each conjugation reaction. Conjugated antibodies were separated from the unconjugated fluorephores by passing through the purification resin and column provided in the kit. Antibodies were conjugated to either Alexa 488 (green) or Alexa 594 (red) fluorophores. The conjugated antibodies were diluted to appropriate concentrations in PBS before injection. The following amounts were injected per oocyte of the various fluorescent antibodies: 10 nl of 0.2 mg/ml Alexa 488 anti-Aur B; 10 nl of 0.5 mg/ml Alexa 594 anti-Aur B; 20 nl of 1 mg/ml of Alexa 594 anti-pMLC.

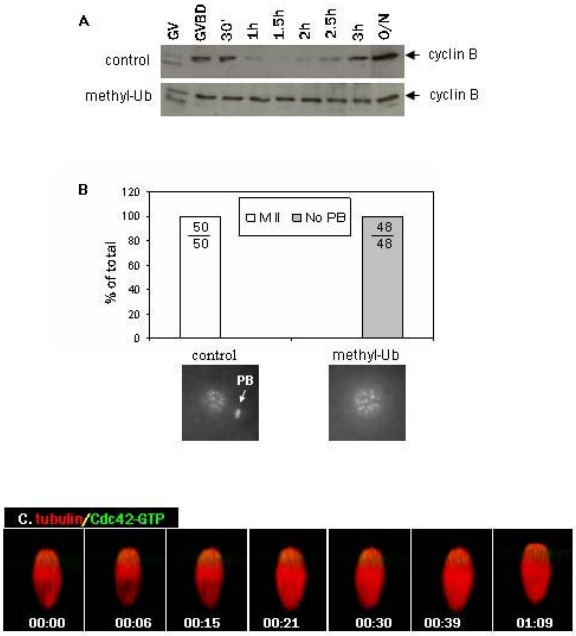

Figure S1

Figure S1. Methyl-Ubiquitin Inhibits Anaphase and Inhibits Cdc42 Activation
(A) Control oocytes (upper panel) and oocytes injected with methylubiquitin (500 ng per oocyte, lower panel) were treated with progesterone and withdrawn at the indicated time for cyclin B2 immunoblotting. GV represents oocytes not treated with progesterone. All time points refer to time after GVBD. Note the degradation of cyclin B in control oocytes but not in methylubiquitin-oocytes.
(B) Control oocytes and methylubiquitin-injected ocoytes were treated with progesterone and fixed 3-4 h after GVBD for chromosome analyses, as in Figure 1B.
(C) A series from 4D movie of an oocyte injected with methylubiquitin, together with rhodamine-tubulin (red) and eGFP-wGBD (active Cdc42, green). Note the lack of Cdc42 activation.

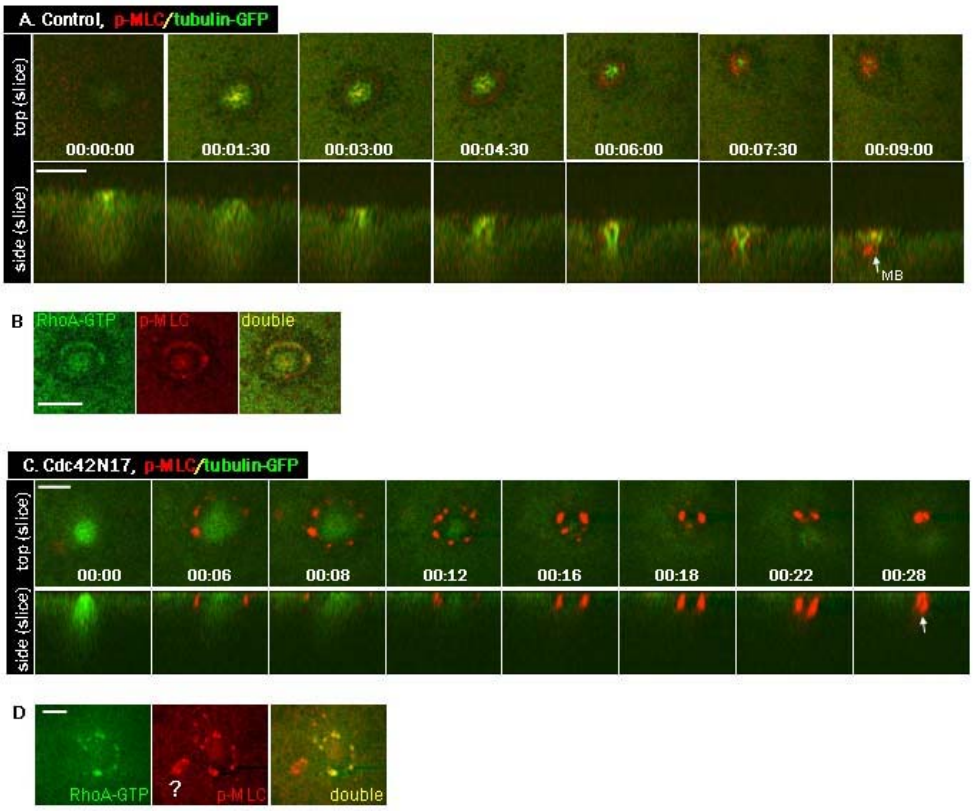

Figure S2

Figure S2. RhoA Contractile Ring Contains Active Myosin Light Chain

To determine whether the RhoA zone is also associated with myosin-2, we chemically coupled a fluorophore (Alexa 594) to antibodies against phosphorylated myosin light chain (pMLC), a known component of the contractile ring (Pelham and Chang, 2002), and employed these fluorescent antibodies to track endogenous p-MLC (Benink and Bement, 2005).

(A) A series from a 4D movie of a control oocyte depicting microtubles (β5-tubulin GFP mRNA) and p-MLC (Alexa 594 anti-p-MLC). The p-MLC signal could be seen forming

a contractile ring (00:01:30) which closed quickly (00:09:00). The midbody is indicated by an arrow.
(B) A single time point of a control oocyte injected with eGFP-rGBD and Alexa 594 p-MLC, indicating complete overlap between the two probes at the contractile ring
(C) A series from 4D movie of an oocyte injected with Cdc42N17 mRNA, together with β5-tubulin GFP mRNA and Alexa 594 anti-p-MLC. The p-MLC signal was much more prominent, reminiscent of the more prominent RhoA signal seen in these oocytes, and similarly constricted and closed.
(D) A single time point of an oocyte injected with Cdc42N17, together with Alexa 594 anti-pMLC and eGFP-rGBD, indicating the complete overlap between active RhoA and pMLC. Non-specific Alexa 594 anti-pMLC aggregates were sometimes observed (indicated by a question mark).

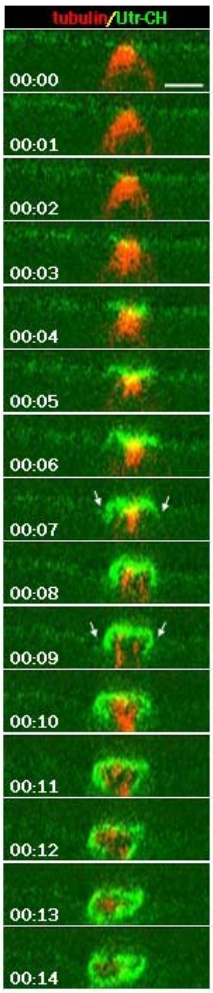

Figure S3

Figure S3. Membrane Curvature During Polar Body Emission

To directly demonstrate membrane curvature during polar body emission, we employed GFP-Utr-CH (Burkel et al., 2007) to visualize cortical F-actin in relationship to the polar body. Prior to polar body emission, the cortex is flat and uniformly labeled with GFP-Utr-CH (00:00). An F-actin cap first appears at the spindle pole-cortex contact site, reminiscent of the Cdc42 activity cap. This F-actin cap then starts outpocketing, creating a bulge representing the protruding polar body (00:07, arrows). Within a few minutes, the F-actin encloses, signifying the completion of polar body emission.

Supplemental References

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