Supplemental Materials

Molecular Biology of the Cell

Field et al.

Supplementary Material

Figure Legends for Supplementary Figures

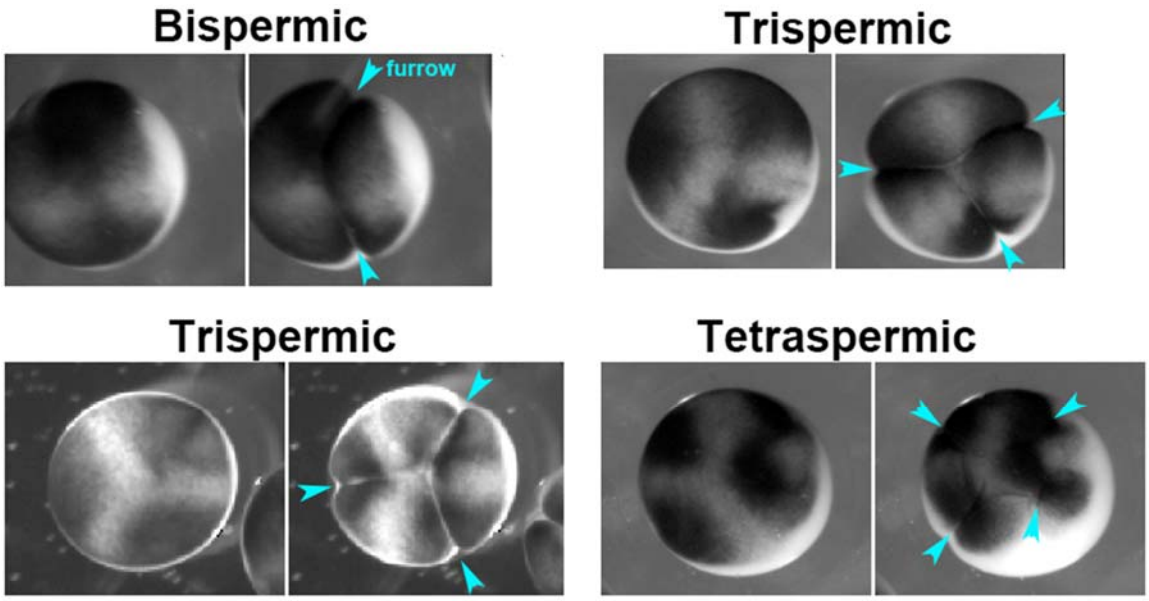

Figure S1. Cleavage patterns in polyspermic eggs
Four examples of showing surface appearance of polyspermic eggs before and after initiating cleavage (taken from time-lapse movies). Before cleavage, cortical pigment concentrates in dark patches over each centrosome-nucleus complex. Cleavage planes bisect these dark patches, because they bisect the position of metaphase spindles. One cleavage furrow initiates per sperm. Furrows tend to fuse if they align, for example in the two furrows in bispermic eggs usually fuse. These images are similar to those previously reported by (Render and Elinson, 1986).

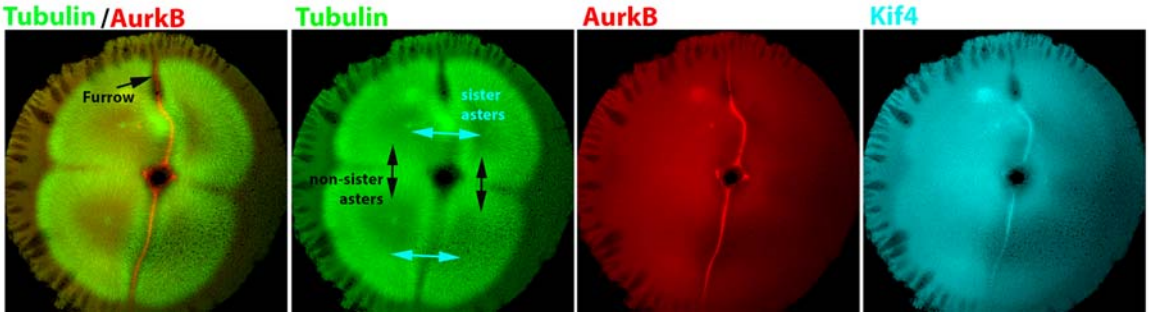

Figure S2. Kif4A co-localizes with Aurora B kinase in sister zones
An example of a bispermic egg fixed at early cleavage (furrow is ingressing from above the plane of focus). Note that Kif4A co-localizes with AurkB and does not localize to interaction zones between non-sister asters.

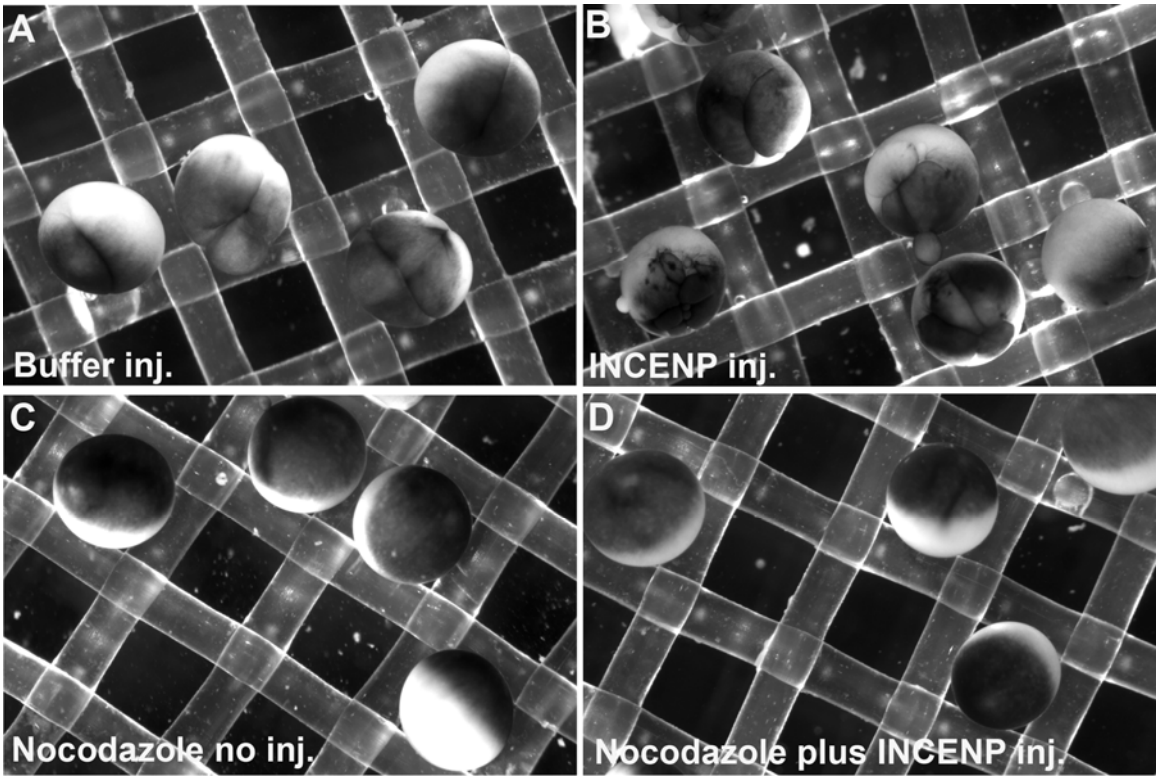

Figure S3. Microtubules are required for normal and ectopic furrow formation
Monospermic fertilized eggs were injected with buffer (A) or an activating anti-INCENP antibody (B, D) or not injected (C). Eggs in panels C and D were fertilized, dejellied and then incubated in 0.1X MMR with 5% Ficoll containing 40μm nocodazole prior to injection. In this experiment, 5/6 buffer injected eggs formed a single furrow (A), while 6/6 anti-INCENP injected eggs formed multiple ectopic furrows (B). Eggs incubated in nocodazole do not initiate any furrows (C, D) 0/6 eggs each). Images shown at 110 min post fertilization from the time-lapse record.

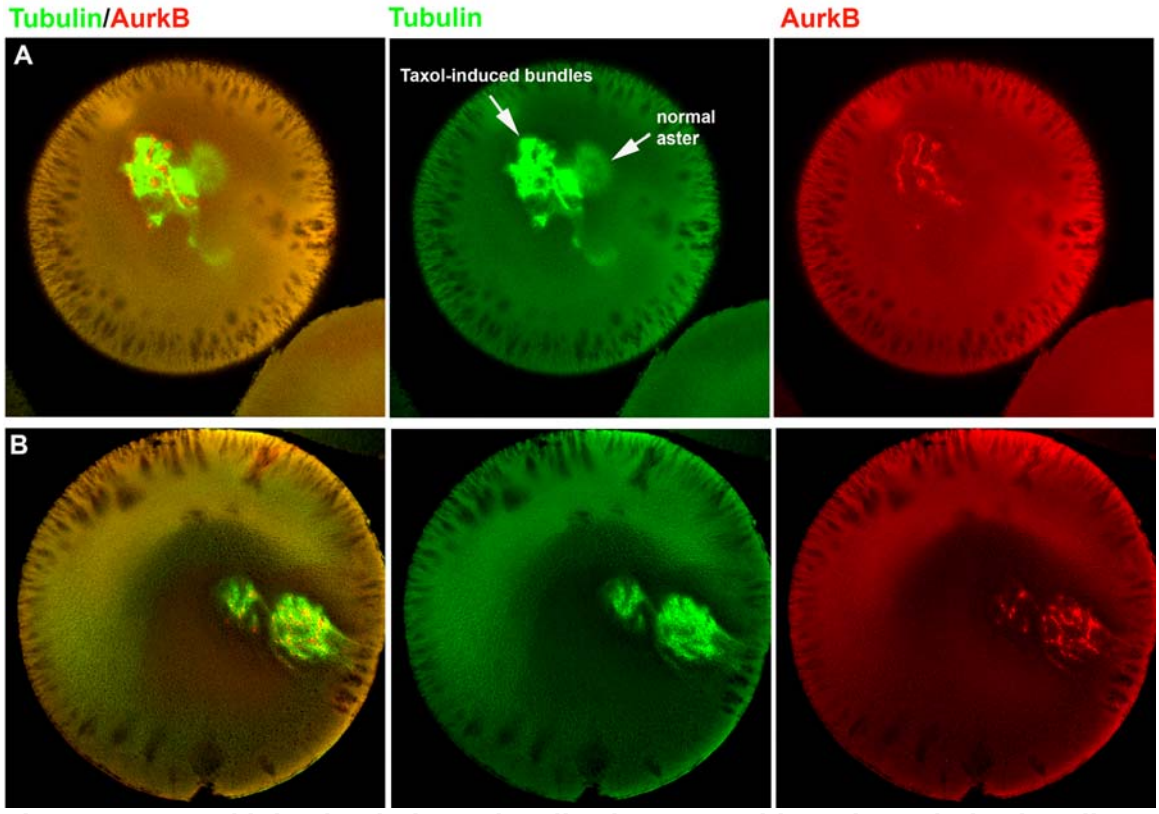

Figure S4. Taxol injection induces localized, CPC-positive microtubules bundles
Two different monospermic eggs injected with taxol (50μM in water). Note dense microtubule bundles that recruit AurkB to foci that only partly overlap with the microtubule density. The bundles are tightly localized in the egg, presumably close to the injection site. In (A) a relatively normal aster can be seen nearby (arrow), showing the localized nature of the taxol effect. The morphology of the taxol-induced aggregates with focal CPC recruitment is reminiscent of bipolar, taxol-induced assemblies in egg cytosol, and in that case the CPC localized to bundle plus ends (Mitchison et al., 2013).

TABLE S1 Furrow ingression in injected monospermic eggs

Injection	**Total # eggs**	**Eggs with single furrow**	**Eggs with multiple furrows[1]**	**Furrow at injection site[2]**	**No cleavage[3]**	**% multiplefurrows**
Anti-INCENP IgG	55	0	50	0	5	100%
Anti-INCENP IgG + Nocadazole[4]	6	0	0	0	0	0%
Anti-MCAK IgG	23	1	19	0	3	95%
Buffer Control	26	20	0	2	4	0%

Table S1. Furrow ingression ininjected monospermiceggs

[1]Ectopic furrows formed at the same time as normal furrows in controls. [2]Small additional furrowoccasionally formed at the injection site, especially when the injection scar was large. These eggswere not scored as multiple furrows.[3]Presumably these eggs were damaged by the injection, and were excluded from the total.[4]Eggs were treated with 40 μMnocodazole after fertilization and before anti-INCENP injection to depolymerize microtubules. This blocked all furrowing. Note: this figure provides quantitation for experiments summarized in Figure 3.

TABLE S2 Furrow ingression and ectopic CPC recruitment in injected and fixed monospermic eggs

Injection	**Total # Eggs**	**Single furrow**	**Multiple furrows**	**Ectopic AurKB localization[1]**	**% multiple furrows**
Anti-INCENP IgGfixed ~90 min post-fertilization[2]	10	0	0	0	Fixed before furrow initiation[2]
Anti-INCENP IgGfixed ~110-120 min post-fertilization[2]	11	0	11	11	100%
Anti-MCAK IgG fixed ~112-125 min post-fertilization[2]	19	2	16	17	84%
Buffer control fixed ~110-125 min post-fertilization[2]	11	9[3]	0	0	0%

Table S2. Furrow ingressionand ectopic CPC recruitmentin injected and fixed monospermic eggs

[1]AurkB localized ectopically to MT bundles at the periphery of asters. In some cases AurkB-positive bundles were present at the shared boundary between two aster-like arrays, and were presumably anti-parallel. In others they were present at the periphery of single asters, and were presumably parallel. See Fig 3 for examples. [2]Eggs were injected at ~70min post fertilization,at or shortly after 1st mitosis. They were fixed either at ~90min, when asters were still small, or at 110-125 min, when asters were large and cleavage had initiated in mosteggs. Ectopic localization of AurkB and formation of additional asters were only observed at the later time points.[3]Two eggs had no furrow; one was defective and one was an early anaphase. Note: this figure provides quantitation for experiments summarized in Figure 3.