Total Cellular Tubulin and Mitotic Spindles

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Developmental Biology
30 November 2009

Introduction

In this study, sea urchin embryos were observed at the blastula phase of development. The hypothesis for the experiment asserted that under fluorescent light, the brightness of the cytoplasm of stained cells undergoing mitosis indicates that the total tubulin within the cell is not all contributed to the mitotic spindle. Tubulin is necessary for the formation of mitotic spindles, but not all for cytoplasmic microtubules (Martin, Osmani, and Oakley 1997). Therefore there may be some remaining tubulin in the cytoplasm of replicating cells, unless it is all used in the creation of the spindles.

Unlike mitotic spindles, microtubules within the cytoplasm of cells contain phosphorylated tubulin. Microtubules within cells undergoing mitosis contain “soluble tubulin fraction” instead (Fourest-Lieuvin, Peris, Gache, Garcia-Saez, Juillan-Binard, Lantez, and Job 2006). Studies show that γ-tubulin is required for the activation of spindle pole bodies (Masuda and Shibata 1996) and plays an important role in the synthesis of alpha and beta tubulin, the components of microtubules (Zhou, Shu, and Joshi 2001). Therefore it is probable that not all of the tubulin within a cell is used to create mitotic spindles during replication.

The organism used in the experiment was *Lytechinus pictus*, or the White Sea urchin. Sea urchin gametes can be easily harvested and fertilized to create embryos that are observable at many stages of development. In the experiment, sea urchin late blastulae were fixed and exposed to the immunofluorescent stains Hoechst and the anti-alpha tubulin antibody FITC-DM1A.

Materials and Methods

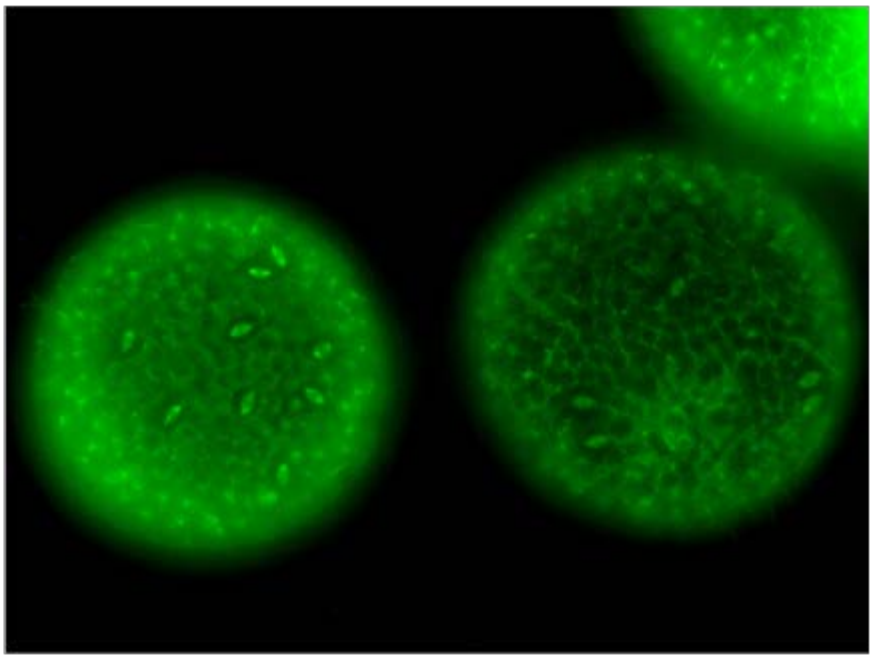
Sea urchin blastulae were initially fixed using cold methanol (MeOH) and then later rehydrated with phosphate buffered saline with Triton X-100 (PSB-T). 1% protamine sulfate was used in mounting the fixed embryos onto coverslips. The stains used included Hoechst staining solution and FITC-DM1A anti-alpha tubulin antibody solution. Bovine serum albumin (BSA) was the buffer used to block the binding of antibody stains to non-specific proteins. Finished coverslips were sealed onto slides with VALAP. Fluorescent imaging was done with a NIKON E400

epifluorescence microscope with standard Hoechst, FITC, and Rhodamine fluorescent settings, using Spot Advanced on a Spot Insight camera from Diagnostic Instruments. All images were taken with a fluorescent objective at 40x magnification.

The procedure carried out followed Professor Robert Morris's "Immunofluorescent staining of sea urchin embryos, MeOH fixation" protocol developed in consultation with Drs. J. Henson and B. Shuster, MBL, in the summer of 2008. Changes to this procedure include using 1% protamine sulfate in place of polylysine in order to make the coverslip surfaces cationic. Also, incubations lasted a shorter period of time – about 20-30 minutes average per incubation.

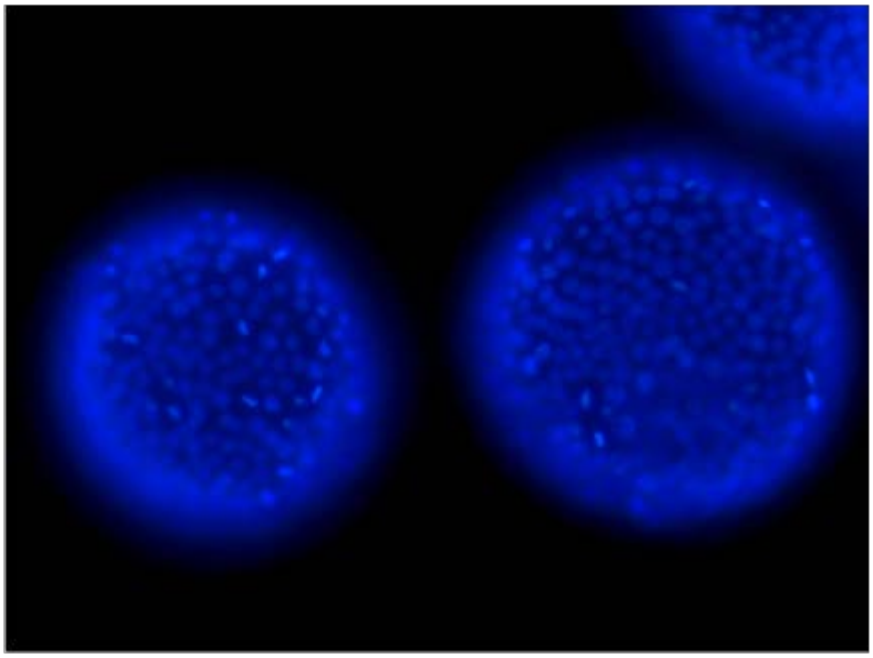
Results

The FITC green fluorescent images show the many cells of the sea urchin blastulae, some of which are undergoing mitosis. In those that are replicating, the mitotic spindle is clearly visible. The images of blue fluorescence from the Hoechst DNA staining show the same cells undergoing mitosis within each blastula, as their DNA is compact and appears bright.



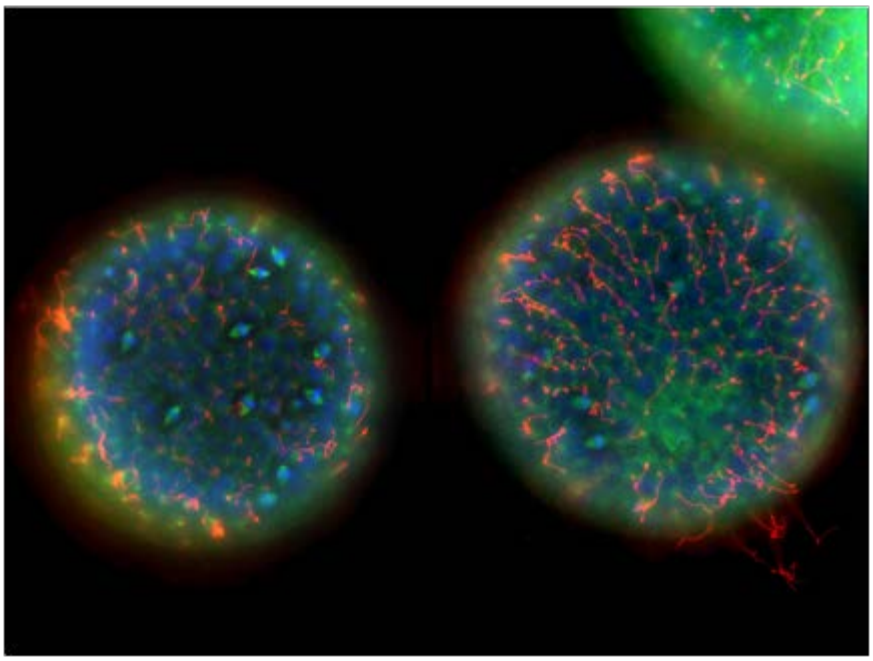
FITC green fluorescence of sea urchin blastulae at 40x

Within both blastulae, mitosis is occurring. The microtubules and mitotic spindles glow green and appear brighter when in greater concentrations.



Hoechst blue fluorescence of sea urchin blastulae at 40x

The DNA of those cells within each blastula appears brighter because the DNA is more condensed than in cells in interphase.



overlay of three fluorescent images (Hoechst, FITC, and Alexa546 GAM (not used for hypothesis)) of sea urchin blastulae at 40x

The layered FITC and Hoechst fluorescent images confirm which cells are undergoing mitosis.

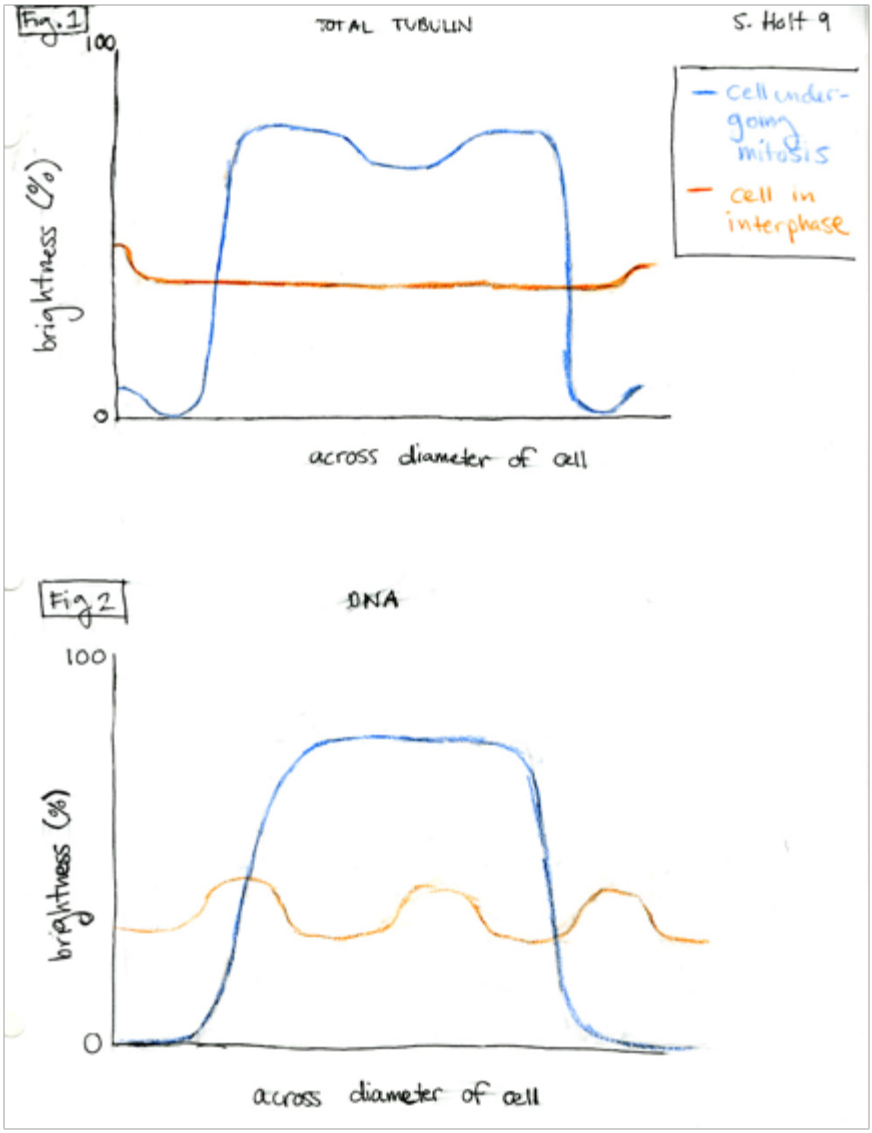


Figure 1 depicts the average percentage of brightness of the total tubulin when looking across the diameter of the cells. If a cell was undergoing mitosis, the diameter measured went across the axis in which cytokinesis would occur. In cells undergoing mitosis, brightness is concentrated in the mitotic spindles. The cytoplasm remains dark with the exception of the plasma membrane, which exhibits a slight brightness. Cells in interphase also have this brightness in their plasma membranes, but it is brighter than cells that are replicating. The cytoplasm of cells in interphase exhibits a slightly dimmer brightness than that of their plasma membranes.

Figure 2 shows that the brightness of DNA in cells that are replicating is much greater than the DNA of cells in interphase. Cells in interphase have regions where brightness is greater and these regions are located towards the center of the cell.

Discussion

The blue DNA fluorescent image supports the claim of which cells are undergoing mitosis. The cells with green

mitotic spindles correspond with the same cells with a bright cluster of condensed DNA, a clear indication of mitosis. The overlay fluorescent image confirms that these cells are the same, as the spindles and condensed DNA are contained in the same cells. The green fluorescence in the very center of the cell is slightly dimmer because it the site where the chromosomes are beginning to split and therefore where the spindles are separating them. The waviness of the DNA brightness graph of cells in interphase is due to the decondensed and delocalized DNA within the nucleus of each cell.

Due to the presence of fluorescent brightness outside of the mitotic spindle in replicating cells of sea urchin blastulae, the hypothesis is supported. The total tubulin in a cell is not completely used up by the formation of the mitotic spindles. The brightness around the plasma membrane of the replicating cells shows that there is tubulin still present there. A small fraction of total cellular tubulin is found to be an integral protein in the plasma membrane. Integral proteins include those that "do not penetrate the bilayer very far but are membrane-bound by a lipid modification." This tubulin may differ slightly from cytoplasmic tubulin (Wolff 2009). This experiment proposes that the integral tubulin remains embedded in the plasma membrane of a cell during mitosis. However, the data suggest that some of the tubulin around the plasma membrane does leave, as the plasma membranes of replicating cells are dimmer than those of cells in interphase. Nonetheless, integral tubulin must continue to play an important role within the replicating cell if it does not join the tubulin of the microtubules in depolymerizing in order to form the mitotic spindles. However, this specific role during mitosis still needs to be investigated. Future experiments involving multiple species and several stages of embryonic development could give possible insight into the specific functions of the integral tubulin in plasma membranes during mitosis.

The main error of the experiment was that there was no real control for the success of the fluorescent staining. The negative control mistakenly was given doses of the stains as well. However, the fluorescent images appear to be quite successful in comparison to images taken from similar experiments performed at approximately the same time.

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