

# From the Archive

## Centrosome choreography

Depending on the position of the mitotic spindle, a dividing cell can split evenly or unevenly, lengthwise or down the middle. As a graduate student at the MRC Laboratory of Molecular Biology in Cambridge, England, Anthony Hyman showed how the centrosomes' travels set up the division axis in *Caenorhabditis elegans* (Hyman and White, 1987).

Hyman was rummaging through the literature when he stumbled across the question of how the cell division axis gets set up. Everyone assumed that the centrosomes determined the positions of the spindle poles, but nobody knew how. His test system was the fertilized worm egg, and the AB and P<sub>1</sub> cells that result from its first cleavage. These cells behave differ-

ently from each other. Divisions in the AB lineage are symmetric, and each occurs at a 90-degree angle relative to its predecessor. Consistent with this change in direction, Hyman (now at the Max Planck Institute of Molecular Cell Biology in Dresden, Germany) found that in AB cells the newly duplicated centrosomes migrated to opposite sides of the nucleus before mitosis. This effectively spun the axis of the cell around.

In the P<sub>1</sub> lineage, by contrast, divisions are asymmetric but the cells always part along the same axis. The same migration of duplicated centrosomes occurred in P<sub>1</sub> cells, but then the nucleus rotated 90 degrees, dragging the centrosomes with it. Nobody had observed this rotation before, but Hyman wasn't surprised.

"At that stage, you are too young and naive to be surprised," he says. Immunofluorescence revealed nets of microtubules stretching from the centrosomes to the cell cortex, suggesting that these fibers helped position the structures and turn the nucleus.

Hyman tackled this issue in a follow-up study, using a laser to slice the microtubules between either of the centrosomes and the cell's anterior cortex (Hyman, 1989). In normal cells, the centrosomes are equally likely to turn toward the cell's anterior. But in lasered cells, the unzapped centrosome always rotated in that direction. Hyman concluded that one spot on the anterior cortex was hooking the centrosome's microtubules and reeling in the centrosome, thereby turning the nucleus.

Other workers, however, postulate a different mechanism based on recent discoveries about the protein LET-99, which girdles the egg (Tsou et al., 2002, 2003).

According to this hypothesis, microtubules from around the cortex are tugging on the nucleus, but LET-99 weakens the pulling force from some parts of the cell, and the resulting imbalance causes the nucleus to turn. What exerts the force on the nucleus and centrosomes remains uncertain. The proposed pulling site on the cortex holds an abundance of dynein (Waddle et al., 1994), which suggests that this molecular motor might provide the power.

Recent work suggests that centrosome movements are linked to another intracellular migration: the spindle's shift toward the posterior end of the cell during anaphase, which sets up an unequal division. Heterotrimeric G proteins help get the spindle moving toward the cell's rear end in *C. elegans*, *Drosophila* (Schafer et al., 2001), and vertebrates (Du and Macara, 2004), and one G protein component also controls centrosome rotation (Gotta and Ahringer, 2001). **JCB**

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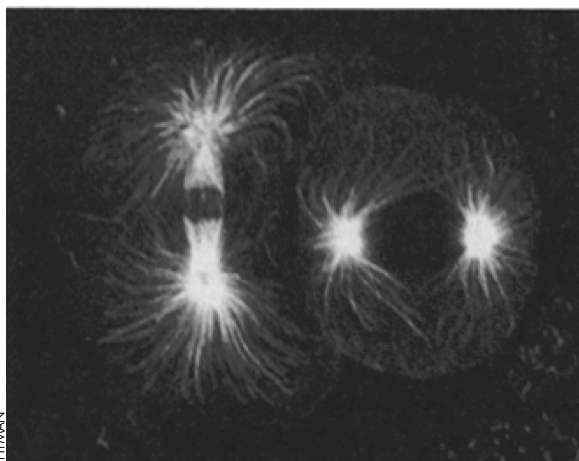
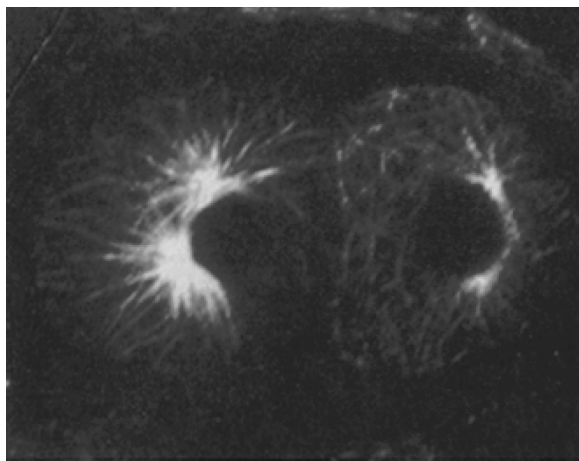
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Tsou, M.-F. B., et al. 2002. *Development*. 129:4469–4481.

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**Tony Hyman investigates how centrosome movements are choreographed, and how they determine the division axis.**



HYMAN

The spindle of the P<sub>1</sub> cell (right) rotates in a 2-cell worm embryo.

## Passenger proteins check in

**D**espite their name, the passenger proteins aren't just along for the ride during mitosis. They are busy helping control the attachment of spindle fibers to the chromosomes and ensuring that the cell splits after the chromosomes part.

### INCENPs' many locations

But it was the proteins' seemingly bizarre movements that first caught the eye of William Earnshaw (now at the University of Edinburgh in the UK) and his colleagues in 1987. Previous work had demonstrated that sister chromatids can separate even when researchers cut the microtubules that form the spindle, suggesting that the centromere might house motors that propel the chromosomes. But cell biologists knew little about the centromere's architecture. In 1985, Earnshaw's lab snared the first three of the structure's components, which they dubbed the centromere proteins, or CENPs (Earnshaw and Rothfield, 1985). Using antibodies against the chromosomes' protein scaffold, Carol Cooke then identified a pair of proteins that cluster on the centromere. The Edinburgh team called this pair the inner centromere proteins, or INCENPs (Cooke et al., 1987).

**Carol Cooke and William Earnshaw identify the first passenger proteins and catalogue their strange movements.**

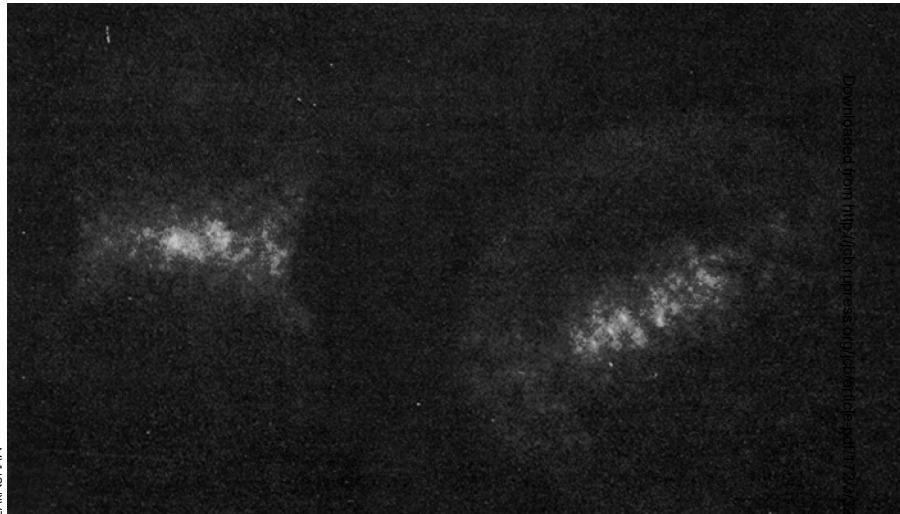
Earnshaw and colleagues could see INCENPs clinging to the arms and centromere in mitotic chromosomes. They took a closer look, treating cells so that the chromosomes were spread-eagled into the textbook "X" shape. They saw that some INCENPs adhered to the centromeres internal to the CENPs at the last points of contact between sister chromatids.

Biochemical tests indicated that the INCENPs clung tightly to the chromosome's protein scaffold, so the researchers got a jolt when they tracked the proteins through mitosis. At the beginning of anaphase the INCENPs appeared to jump ship. Instead of following the chromosomes as they slid apart to the poles, the proteins festooned the microtubules in the middle of the mitotic spindle. And some snuggled up to the cell membrane at the point where the cleavage furrow later squeezes the cell in two. "Nobody had seen a chromosomal protein change its position that dramatically," says Earnshaw. He and his colleagues had identified the first passenger protein (the two INCENPs turned out to be splice variants of the same molecule).

### Many places, many functions

INCENP concentrated at the site of presumptive cleavage furrow formation before myosin appeared there (Eckley et al., 1997), making it tempting to speculate that it was being

dropped at the site after hitching a ride on the chromosomes as a "passenger." But earlier functions were still under consideration. Earnshaw had found that cells injected with anticentromere antibodies failed to align chromosomes during metaphase and, although they kept going through mitosis, they did so with extremely defective spindles (Bernat et al., 1990). "This led me to think that the chromosomes must normally 'give' something to the spindle in metaphase that helped stabilize it in anaphase," he says. The candidate for that something was INCENP.



INCENPs cluster at the middle of each cell even at late- (left) or mid-anaphase (right).

Later work showed that INCENP partners with three other passenger proteins—Aurora-B, survivin, and borealin—to form the chromosomal passenger kinase complex. This complex targets many proteins in the cell including histone H3, which acquires phosphate tags as the chromatin condenses at the onset of mitosis. The passenger complex also helps to fasten microtubules to the centromeres and to choreograph the separation of the two daughter cells. Just last year, the labs of Earnshaw and Hironori Funabiki (Rockefeller University, New York, NY) uncovered borealin (Gassmann, 2004), also known as Dasra (Sampath et al., 2004), and showed that it's crucial for correctly attaching microtubules to the centromeres, stabilizing the spindle, and completing cell division. When Earnshaw and colleagues watched INCENP flitting about in 1987, they were seeing the protein complex on the job. **JCB**

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