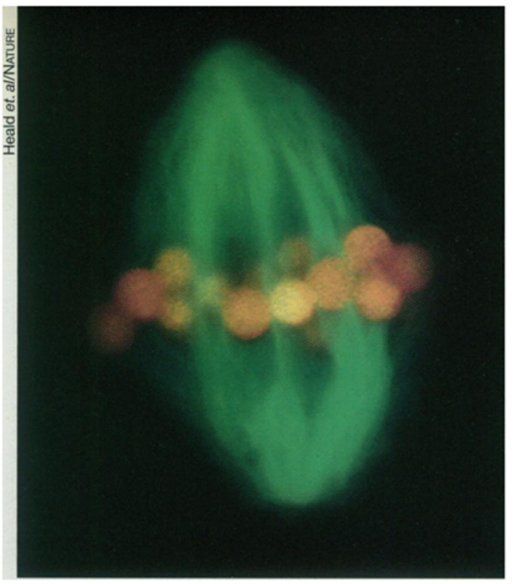
Mitotic Mischief

Can cells divide without chromosomes?

By JOHN TRAVIS



DNA-coated beads (yellow) assemble a mitotic spindle (green) indistinguishable from those in normal dividing cells.

Consider the role of the corpse at a funeral. Though the reason for the often elaborate proceedings, the corpse can do nothing to control the events taking place around it. In the end, its fate rests in the hands of the pallbearers.

In 1961, when discussing the role that chromosomes play in the splitting of a single cell into two, the late Daniel Mazia drew an analogy with corpses at funerals. At first blush, the idea of these threads of DNA as inactive participants in cell division seems improbable. After all, before a cell divides, its chromosomes must duplicate, separate into two sets, and move to opposite sides of the cell.

Do chromosomes, however, actually direct the drama of the dividing cell? Or are they, like the corpse, central but ultimately passive characters?

Two research groups, both of which describe their work in the Aug. 1 NATURE, have now made dramatic attempts to resolve this issue. One team observed what happens to the assembly of the spindle, an intricate structure needed for cell division, when plastic beads covered with DNA stand in for chromosomes. The second group performed the even more daring feat of asking cells to perform the final acts of cell division without chromosomes or even any substitutes.

The ability to divide properly may be the most important skill that cells possess. Without thousands upon thousands of perfect cell divisions, for example, the fertilized egg could not transform itself into a healthy baby.

While some cells, such as the brain's neurons, rarely divide in adults, biologists believe that every cell can double its contents and split in two if given the proper signals. Indeed, many cells spend the majority of their lives preparing for cell division. During interphase, the longest stage of the cell's life cycle, the cell stockpiles proteins and other crucial molecules. It also copies its DNA. With that groundwork laid, the cell awaits the main act in the division process: mitosis.

Mitosis creates two nuclei from the cell's single nucleus. Its name derives from the Greek word for thread, because the first visible signs of mitosis under most microscopes are the disappearance of the boundary around the nucleus and the concentration of the nucleus' normally diffuse DNA, along with some proteins, into threadlike chromosomes.

"Cell division is a very dramatic phase in the life of the cell. When you get into mitosis, everything changes—the nuclear envelope breaks down, the chromosomes condense, the spindle forms," says Jeremy Hyams of University College London.

The spindle, one of the most photogenic, though temporary, cellular structures, is a football-shaped scaffold of long hollow rods. Called microtubules, the rods form from the protein tubulin. The spindle's microtubules line up the duplicated chromosomes in a plane across the center of the cell. This arrangement ensures that when they later disjoin and move apart, each half of the cell receives an identical set of chromosomes. The spindle is also necessary for this separation to occur, though its exact role is still hotly debated.

How does the elaborate spindle form? Biologists have long held that chromosomes were vital to this essential component of the cell division process. Most current theories of spindle assembly focus on the interplay between the centromeres, which are specific DNA sequences on chromosomes, and the centrosomes, sites of microtubule construction outside the nucleus.

In one explanation, known as the search-and-capture model, growing microtubules radiate out from two centrosomes at opposite ends of a cell. Complexes of proteins at the centromeres then capture and stabilize the microtubules to create the spindle.

This model has derived support from many studies over the years, including ones in which chromosomes are removed from living cells. As the number of chromosomes decreases, so does the number of microtubules in the spindle, explains R. Bruce Nicklas of Duke University in Durham, N.C. This particular finding, however, doesn't distinguish what parts of the chromosomes are used to assemble the spindle.

Investigators from the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany, have crushed dozens of eggs in an attempt to resolve that issue. Specifically, they are working with the extracts of eggs from the African frog, *Xenopus laevis*. With such extracts, the scientists can monitor in a test tube the same cell cycle stages, including spindle assembly, that occur in frog cells.

Recently, Rebecca Heald and her colleagues at EMBL, including Anthony Hyman and Eric Karsenti, came up with the idea of removing the centrosomes and the chromosomes from the frog egg extracts. They replaced the chromosomes with beads coated with random sequences of DNA. Though the beads served as substitute chromosomes, the test-tube system lacked several of the elements that the proponents of the search-and-capture model thought necessary for spindle assembly. Among the missing elements were kinetochores, the complexes of protein at the chromosomes' centromeres.

The investigators found that the genetic material on the beads condenses as if in a chromosome and induces the formation of microtubules. More important, within 90 minutes, the microtubules gather into a spindlelike structure that aligns the beads in a plane at the middle of the bipolar assembly.

"Somehow, the microtubules are organized from a random array into an organized array. What we've shown definitively, at least in this system, is that search and capture is not the mechanism [of spindle assembly]," says Hyman. "We've shown that you don't need kinetochores or centrosomes."

This unexpected finding has caught the imagination of cell biologists. "You can build a perfectly good spindle. That's a real surprise. I find it remarkable, actually," says Hyams, who wrote a commentary accompanying the report.

"The formation of the spindle is indisputable. The images look quite convincing," adds J. Richard McIntosh of the University of Colorado in Boulder.

Additional experiments by the EMBL group have suggested an explanation for how their spindles arise. Microtubules first assemble near the chromatin-coated beads. Dyneins, members of a family of proteins known as motor molecules, then attach to the microtubules and travel to one end, known as the minus end. Leaving the plus ends at the center of the cell, the dyneins pull the minus ends to either side of the beads. Since each motor molecule attaches to many microtubules, the minus ends of the rods collect at two sites on opposite sides of the beads and a bipolar spindle appears.

"What we're saying is that a driving force behind spindle assembly is microtubules and motors," comments Hyman.

Based on other experiments, Hyman and his colleagues believe the centrosomes determine where the minus ends of the microtubules will normally concentrate. "Centrosomes will influence spindle assembly, if they're there. Centrosomes are dominant, but they're dispensable," explains Hyman.

The researchers aren't quite ready to accept Mazia's extreme idea that chromosomes do nothing to direct mitosis. The kinetochores, for example, are vital to the later segregation of the two sets of chromosomes, say the researchers.

High on the EMBL group's research agenda is determining how the DNA-covered beads induce microtubules to form and whether their proposed mechanism for spindle assembly is universal among dividing cells.

McIntosh suggests that spindle formation is so important that cells have more than one method of doing it. "There are multiple ways of setting up spindlelike structures, and certain cell types will emphasize one approach relative to another," he contends.

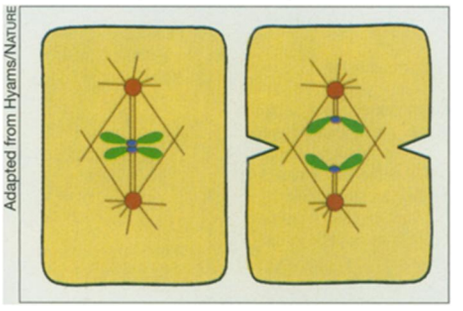
While the researchers in Germany worked with artificial chromosomes in the test tube, Nicklas and Duke coworker Dahong Zhang studied what happens to cell division when they removed all the chromosomes from a cell and made no replacements.

More than 3 decades ago, Nicklas began using a micromanipulator, a fine glass needle controlled by a joystick, to study chromosomes in living cells. The needles can probe the interior of certain cells, such as the immature sperm cells of grasshoppers, without causing damage.

Nicklas had initially planned to measure the forces that move chromosomes around in the dividing cell, but he got sidetracked. "One day I pulled a chromosome off the spindle, and it popped right back on. I thought, `My gosh, this is neat.' This proved to be so interesting that we didn't get around to measuring forces for 20 years," laughs Nicklas.

In the last few years, he and Zhang have been pulling chromosomes completely out of cells. Recently, the two performed this trick on chromosomes that were lining up at the center of assembled spindles. In a normal cell, after the chromosomes align at the middle of the spindle, they split into two sets and then slowly move apart. This movement marks the stage of mitosis known as anaphase.

What triggers anaphase has been a subject of some debate among cell biologists. One faction holds that a molecule stemming from the separation of the joined chromosomes actively signals the start of anaphase. Yet anaphase seems to occur in the chromosomeless cells created by Nicklas and Zhang. A gap appears at the equator of the spindle and slowly widens toward the poles, the same phenomenon observed when chromosomes are along for the ride.



In this simplified diagram of mitosis, the spindle's microtubules (black lines) position a copied chromosome (green) at the middle of the cell. The two identical chromosomes, joined by kinetochores (blue) then separate and move to the cell's centrosomes (red). The cell finally splits in two.

The chromosomeless cells have also explained more fully how a cell knows when it is time for anaphase. In previous experiments with cells that had chromosomes aligned on the spindle, investigators had shown they could forestall anaphase indefinitely by using a micromanipulator to keep a single chromosome out of place. "The record is 4.5 hours," says Nicklas.

That finding left open a question. Does a cell proceed with anaphase only when there is the correct number of properly positioned chromosomes or only when there are no chromosomes out of line? Since cells without chromosomes undergo anaphase, the latter idea, already supported by some earlier studies, seems correct.

An important, unresolved issue surrounding anaphase is how the chromosomes travel along the spindle as they move to the poles. One possibility is that the microtubules, which appear to shorten during anaphase, pull their attached chromosomes along like fish hooked on a line.

Another popular idea has the chromosomes, powered by molecular motors, inching along the microtubules, like a person climbing a rope. Since the microtubules seem to be disassembling at the kinetochore, where they're attached to the chromosome, some investigators have proposed that the kinetochores cause this shortening by munching away on tubulin like Pac Man.

While the chromosomeless cells do not provide definitive evidence for either theory of movement, they do suggest that kinetochores may not be necessary. "It looks as if the microtubules are shortening, even in the absence of the kinetochores," notes Nicklas.

To their delight, Nicklas and Zhang found that their micromanipulated cells not only went through anaphase but actually split apart. In this last stage of cell division, the determination of where the cell cleaves is crucial. "You have distributed the genetic material to opposite sides of the cell, but you darn well better get the division of the cell in the right place or all your care in handling the chromosomes will go for naught," explains Nicklas.

Normally, a dividing cell begins to pinch in along the plane where the chromosomes originally lined up on the spindle. Though the spindle and its two poles together can determine this cleavage plane, several researchers have suggested that chromosomes actually mark the location. In support of this hypothesis, scientists have identified several proteins that are shed by chromosomes immediately before they begin to move from the spindle's equator.

The chromosomeless cells offer evidence against this proposal, however. The researchers removed the chromosomes at a point in mitosis long before the proteins are thought to be shed. Yet, says Nicklas, "the site of the cleavage furrow occurred in precisely the right location, midway between the poles of a chromosomeless spindle. The chromosomes are obviously not dictating the site."

The work of Nicklas and Zhang, as well as that of the EMBL group, has certainly forced biologists to reconsider how much direction chromosomes exert on the various stages of cell division.

"They're just two very beautiful sets of experiments that make us think about mitosis in a different way. They're the sort of experiments you would have loved to have done yourself," says Hyams.

While chromosomes no doubt have some influence, he acknowledges, that influence seems far more limited than previously believed. Mazia's provocative image of a corpse at a funeral, Hyams concludes, may not be so extreme an analogy. □