Cell Division Gatekeepers Identified

The stretches of DNA known as kinetochores not only link chromosome pairs to the fibers that separate them in dividing cells but also regulate the timing of that separation

Just as a group of young schoolchildren must all get in line and be counted before they can get on the bus and head home from a field trip, all the chromosomes inside the cell's nucleus must also line up before the cell can divide. The object, of course, is to make sure that no chromosome gets left behind when the daughter cells separate. Otherwise, one daughter cell could wind up with too much genetic material, and the other with too little.

For decades, cell biologists have wondered just how the dividing cell keeps track of its genetic charges. Now a convergence of research in yeast genetics, cell biology, and biochemistry suggests an answer: Molecular nannies called kinetochores keep a sharp eye on the chromosomes' status. These specialized bits of DNA and protein were already known to have a mechanical role in cell division. They attach the duplicated chromosomes to the fibers of the mitotic spindle, which eventually pulls the chromosomes apart. But the new work, some of which was presented last month at the annual meeting of the American Society for Cell Biology in Washington, suggests that the kinetochores are far more than just anchors for the spindle fibers. "The kinetochore is very active in both the mechanics and the control of chromosome movement," says Richard McIntosh, a cell biologist at the University of Colorado, Boulder.

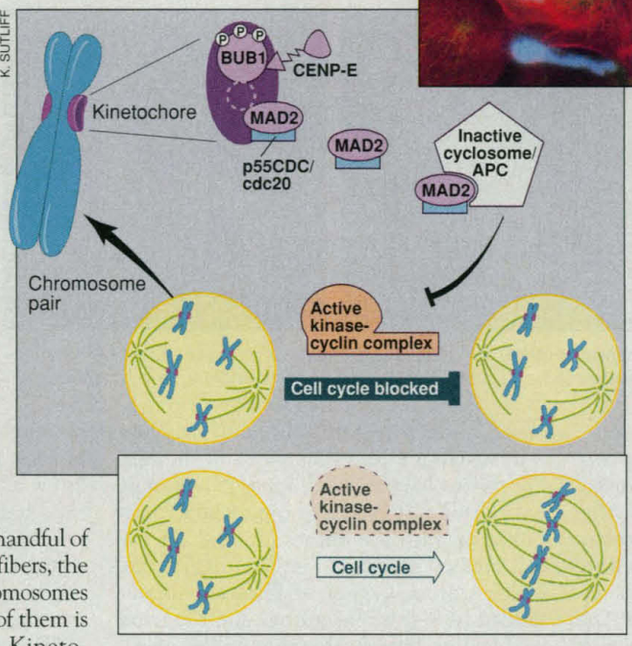
Acting in conjunction with a handful of proteins that tie them to the spindle fibers, the kinetochores make sure that the chromosomes are not pulled apart until every one of them is lined up and attached to the spindle. Kinetochores that have not yet hooked up to the spindle fibers apparently release a protein signal that works with other proteins to put the brakes on cell division. But when the spindle attaches, a protein involved in that linkage somehow disables the wait signal, thereby releasing the brakes on mitosis.

The work is intriguing, says Don Cleveland, a cell biologist at the University of California, San Diego (UCSD), because it adds to recent evidence that the cytoskeleton—the cables, struts, and anchor points inside the cell—doesn't just carry out orders issued elsewhere in the cell. Instead, it is an integral part of the cell's command structure, generating signals of its own. In this case, says Cleveland, "[the work] links the cytoskeleton to the continuation of the cell cycle."

This News report accompanies a special issue on the cytoskeleton that begins on page 509.

Understanding that connection can in turn reveal how the cell cycle goes awry. Errors in chromosome separation can lead to aneuploidy—daughter cells with too many or too few chromosomes—which underlies disorders such as Down syndrome and may play a role in aggressive cancer by causing cells to "go genetically crazy," says McIntosh. "Aneuploidy is a fairly important problem."

C. RIEDER



Checkpoint on. This chromosome pair can't split because MAD2 proteins leaving the kinetochores link to p55CDC/cdc20 and inactivate the cyclosome/APC, which in turn can't break down the kinase/cyclin complex that blocks cell cycle progression. But when microtubules attach to the chromosome, CENP-E changes its interaction with BUB1, thus altering MAD2 and preventing it from inhibiting the APC. It takes just one unaligned chromosome (blue, in inset) to delay the cell cycle.

The current view of kinetochore action began to solidify about 4 years ago when cell biologist Conly Rieder of the New York State Department of Health's Wadsworth Center in Albany decided to test a hypothesis that McIntosh had suggested a few years before. McIntosh wanted to explain why cell division does not occur until every one of the duplicated chromosomes lines up along the cell's midline and attaches to the spindle, which consists of filaments called microtubules that grow into the cell interior from the opposite ends of the cell. "The failure of a single chromosome to attach is sufficient to keep the cell cycle from going forward," Cleveland says.

Some researchers thought that this "checkpoint" in the cell cycle couldn't be released until each chromosome had attached to the spindle fibers and individually given its OK. But McIntosh suggested that it would be simpler if unconnected kinetochores simply gave off a "wait" signal that was somehow silenced by spindle attachment. Although the idea was well received, Rieder worried that no one had demonstrated that there really was such a delay or that it was tied to the kinetochores.

To try to find out, Rieder and his colleagues filmed more than 100 cells as they divided. They found the predicted delay. While the time it took for the chromosomes to line up varied, they always separated 20 minutes after the last one was in position. What's more, Rieder's results indicated that unattached kinetochores are the source of the wait signal: If he destroyed the last unattached kinetochore with a laser, the cell proceeded to divide as if that chromosome pair were already in line. At Duke University in Durham, North Carolina, Bruce Nicklas, by mechanically manipulating chromosome pairs, also showed that waiting is an essential part of the cell cycle. "That led to the universal idea that the action is at the kinetochore," says Andrew Murray, a cell biologist at the University of California, San Francisco.

Another experiment conducted at about the same time strengthened that conviction. Working together, cell biologist Gary Gorbsky of the University of Virginia, Charlottesville, and Nicklas demonstrated that some proteins in the kinetochore change their character,

losing phosphate groups, once the spindle fibers attach. "They were the first to correlate a change in kinetochore chemistry with a change in attachment [dynamics]," says Rieder. "The field entered warp speed."

To find the proteins causing these changes, researchers decided to take a hard look at two sets of genes that yeast geneticists had identified in 1991 as being essential for making that species's chromosomes wait until they all lined up before separating. M. Andrew Hoyt and his colleagues at Johns Hopkins University found one set including three genes that they named *bub1*, *-2*, and *-3*. And Murray found a different set of three, dubbed *mad1*, *-2*, and *-3*. Yeast cells and their chromosomes are too small, however, for researchers to identify where the proteins produced by the genes are concentrated.

Researchers had better luck in the much larger cells of mammals and frogs, where they were able to find and track the equivalents of the yeast proteins. The kinetochore proved to be the center of the proteins' activities. Murray's team found MAD2 on unattached kinetochores of frogs, and Yong Li and Robert Benezra at the Memorial Sloan-Kettering Cancer Center in New York City identified the human MAD2 (*Science*, 11 October 1996, pp. 242 and 246). The human protein, Li and Benezra later found, concentrates at the kinetochore before the chromosomes become attached to the spindle but leaves by the time they are aligned. And Frank McKeon at Harvard Medical School, who identified BUB1 in mice last year, found that that protein also accumulates at the kinetochore during mitosis.

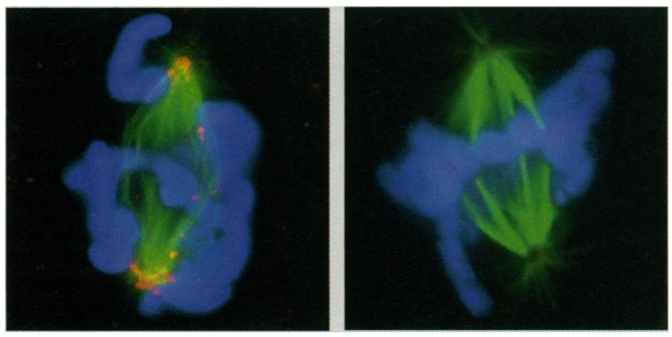
Now researchers are unraveling the molecular choreography—staged at the kinetochore—that enables these proteins and some newly discovered ones to regulate chromosome separation. "All the proteins that have been identified interact in one way or another at the kinetochore," says Gorbsky. "And many of the components are very dynamic, coming on and off the kinetochore."

One of those dynamos is MAD2, which is involved in the wait signal. At first, the high concentration of MAD2 found on the kinetochores until microtubules attach seemed to be necessary for arresting cell division. When Gorbsky injected antibodies that combine with MAD2 and prevent it from functioning into rat kangaroo kidney cells growing in culture, "we had premature separation, when some of the chromosomes were not well connected, or tangled up," he said at the cell biology meeting.

But now it seems that MAD2 does more than simply accumulate on unattached kinetochores. Copies of this protein are also continuously migrating into the cytoplasm, where they somehow broadcast the wait signal throughout the cell. Gorbsky has evidence that in mammals, MAD2 transmits its signal by associating with a protein known as p55CDC, while Murray sees it link up with the equivalent protein in yeast, which is called cdc20.

These duos then apparently head for a large protein complex called the cyclosome or APC (anaphase promoting complex). When active, the complex helps the cell initiate anaphase—the stage of the cell cycle when chromosomes separate and move toward opposite ends of the cell—by catalyzing the breakdown of cyclins and other proteins that otherwise put the brakes on mitosis. For example, the APC likely gets rid of the proteins that glue chromosome pairs together. But the presence of MAD2 and its partners keeps the APC in check, stalling out cell division. "As long as you have free kinetochores, the [wait] signal is continuously generated," says Gorbsky.

Then, as each kinetochore connects to microtubules, the signal from that kinetochore is silenced, until eventually no more wait signals are being generated in the cell. For this to happen, each kinetochore must have some way of recognizing when it has linked up with the mitotic spindle. At the cell biology meeting, Tim Yen of the Fox Chase Cancer Center in Philadelphia described how this recognition and silencing could occur. It seems that the BUB proteins, together with a protein called CENP-E that helps tether the microtubule to the kinetochore, trigger a series of changes along the checkpoint pathway that ultimately shuts down transmission of the "wait" command, at least in human cells.



Dynamic protein. MAD2 (red) concentrates in kinetochores before chromosomes (blue) align, but disperses as the spindle (green) attaches.

J. WATERS AND E. SALMON/UNIVERSITY OF NORTH CAROLINA

Seven years ago, while working together, Yen and UCSD's Cleveland had found that CENP-E, a molecular motor that helps transport cellular components along microtubules, associates with kinetochores. In test tube experiments and in live mammalian cells, they also found evidence that CENP-E plays a role in achieving accurate chromosome alignment during mitosis. For example, when the researchers removed CENP-E from the system, mitosis occurred before the chromosomes had a chance to line up.

Both Yen's and Cleveland's labs have now shown that CENP-E is important for linking kinetochores to microtubules. In addition, Yen's team has tied this protein to human BUB proteins, which were discovered recently in his lab by Gordon Chan and Sandra Jablonski. As chromosomes begin to pair off, the human BUB proteins move to the kinetochore. There, a BUB1 protein, in conjunction with BUB3, links up with CENP-E. "This kinetochore association puts [these proteins] in the right place" for monitoring spindle assembly, says Hoyt.

Yen thinks that when the microtubule links up with the CENP-E, it somehow changes the structure of the molecular motor protein. This change may in turn alter BUB1's activity, possibly by removing phosphate groups from it. Whatever the nature of the change, it apparently leads to the disabling of MAD2, making this protein incapable of binding to p55CDC and shutting down the APC. As a result, mitosis can proceed.

This scenario jibes with new findings in yeast as well. At the meeting, Hoyt's team reported that BUB1 is also an early component of the checkpoint pathway in yeast. That group also finds that BUB1 works with BUB3 while the wait signal is being generated. "Not only is the kinetochore a structural part of the chromosome that contains molecular motors, but you have regulatory components [there], all in the same place," Yen explains.

Of course, McIntosh and others emphasize that there are still gaps in the picture they are developing. They don't know all the molecules involved. Nor have they traced the connections among all the ones they do have in hand. It is unclear, for example, what changes occur in MAD2 as the kinetochore attaches to the spindle or what the links are between MAD2 and the BUB proteins. Moreover, cell biologist Douglas Koshland from the Carnegie Institution of Washington in Baltimore, Maryland, worries that too much emphasis is being placed on the kinetochore. "There is a fair amount of evidence that everything is going through the kinetochore," he says. "But it's not necessarily hard fact" that it is the sole source of the "wait" signal.

Yet kinetochore researchers are encouraged by their glimpses of how some of these molecules do interact. "When you know the players, you can construct hypothetical pathways to test," McIntosh explains. "It's all falling into place."

–Elizabeth Pennisi