Two unsuspected proteins may hold the key to creating artificial chromosomes

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Whitehead Institute scientists report that two proteins once thought to have only supporting roles, are the true "stars" of the kinetochore assembly process in human cells.

The kinetochore is vital to proper DNA distribution during cell division. This finding suggests that scientists may be able to stimulate kinetochore assembly in a process that could lead to new genetic research tools, such as efficient creation of artificial human chromosomes.

"When you understand a process really well, then it can become a tool. And I think this is a nice example of that," says Whitehead Member Iain Cheeseman. "We now fundamentally understand something about the way kinetochore specification and assembly works. And because we understand that, now one could imagine it being used as a tool."

For many years scientists have understood the kinetochore's role in cell division but did not know how its individual parts came together during this process. At the beginning of cell division, the kinetochore consists of a few proteins associated with a chromosome's centromere, which is the section where the arms of an X-shaped chromosome join. As cell division progresses, additional kinetochore proteins attach at the centromere, ultimately forming a complete kinetochore complex consisting of about 100 proteins. At this point, one kinetochore is partially integrated into each lengthwise half of the chromosome, called a sister chromatid; a chromosome's sister chromatids are identical copies of the same piece of DNA.

To distribute the sister chromatids between the two future cells, long protein filaments from opposite sides of the cell reach out, latch onto the chromatids' kinetochores, and begin pulling on them until the sister chromatids split apart. Then, the chromatids are dragged to opposite sides of the cell, ensuring that the future cells will each have a copy of this piece of DNA.

To identify which proteins are necessary for a kinetochore to self-assemble, Karen Gascoigne, a postdoctoral researcher in the Cheeseman lab, positioned three of them on the chromatids' DNA and away from their normal location on the centromere. By moving the proteins away from their normal position, Gascoigne isolated the effects of each protein from potential interactions with the centromere and highlighted the capabilities attributable only to that protein.

The first protein, called CENP-A, is essential for identifying where the kinetochore should locate, and many scientists thought it was vital to kinetochore assembly. However, when Gascoigne moved CENP-A away from the centromere, only a few kinetochore components were recruited to attach onto CENP-A, showing that this protein is not responsible for assembling an entire kinetochore.

When Gascoigne moved the proteins CENP-C and CENP-T away from the centromere, the two proteins attracted almost all of the kinetochore proteins to their location and fostered assembly of a makeshift kinetochore capable of separating sister chromatids.

"So that tells us that these two proteins are essential and sufficient to build the kinetochore even in the absence of CENP-A," says Gascoigne, who reports her findings in the April 29 issue of *Cell*. "Which is unexpected and very exciting. This tells us a lot about how kinetochores are put together in the cell."

This new ability to form a kinetochore anywhere on DNA may be particularly useful for creating artificial human chromosomes. These collections of genes could be used in research to insert new genes into a cell. Currently, scientists insert genes by infecting cells with a virus that haphazardly inserts the DNA into the cells' genomes, a process that can corrupt essential genes and possibly kill cells.

Artificial chromosomes circumvent this potential damage. But their widespread use is thwarted by scientists' current inability to outfit artificial chromosomes with kinetochores. Without kinetochore complexes for those long protein filaments to latch onto during cell division, the artificial chromosomes cannot be passed from the original cells to subsequent generations, meaning that the artificial chromosomes' traits are lost.

By perfecting kinetochore assembly using just CENP-C and CENP-T, Gascoigne is working to overcome this shortcoming in artificial human chromosomes.

"We're interested to see if you can bypass having a centromere by putting in just these two proteins into the artificial chromosome," says Gascoigne. "And then the cell would build the kinetochore itself, which will allow the artificial chromosome to segregate during cell division."

More information: Karen E. Gascoigne (1), Kozo Takeuchi (2), Aussie Suzuki (2), Tetsuya Hori (2), Tatsuo Fukagawa (2), and Iain M. Cheeseman (1). "Induced ectopic kinetochore assembly bypassing the requirement for CENP-A nucleosomes" *Cell*, April 29, 2011

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