Scientists discover link between control of chromosome duplication and segregation

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Before a cell can divide into two, first it must duplicate its genetic material--the DNA packed in its chromosomes. The two new sets of chromosomes then have to be separated from one another and correctly distributed to the resulting "daughter" cells, so that both daughter cells are genetically identical to the original, or "parent," cell.

During cell division, a cellular organ called the centrosome, and a copy of the centrosome, position themselves at opposite ends of the dividing cell. Each centrosome serves as an anchor for a spindle, a complex structure of filament-like tubules that radiates out from each centrosome and connects with special sites called centromeres on the chromosomes. By pulling on the chromosomes, the spindles separate them into two sets, each divided equally into the two emerging daughter cells.

It's crucial that cells duplicate their centrosome only once during each division cycle. Extra copies can result in incorrect distribution of chromosomes, which can lead to genomic instability and cancer. Hence the importance of new research by Professor Bruce Stillman, Ph.D., and his lab group at Cold Spring Harbor Laboratory (CSHL). They have identified a protein molecule that controls the copying of the centrosome in human cells and prevents it from being re-duplicated. Their findings will appear in the February 6th issue of the journal *Science*.

Double duty for Orc1

The molecule shown by Dr. Stillman and his colleagues to control centrosome duplication is Orc1, one of six proteins that comprise the Origin Recognition Complex. ORC, as it is called, is an assembly that attaches to particular sequences within all the DNA in the cell and prepares it for duplication. Recently it had become clear that some ORC proteins might be doing more than jump-starting DNA duplication; the accumulation of extra centrosome copies in cells that lack ORC suggested that some or all ORC proteins might play a role in centrosome duplication as well.

To investigate which of the ORC proteins limit centrosome copying, Stillman and co-investigators Adriana Hemerly, Supriya Prasanth and Khalid Siddiqui, used RNA interference, or RNAi, a technique that uses small pieces of RNA to shut off specific genes. They blocked the production of each of the proteins that combine to form ORC in human cells. Loss of Orc1 alone, the scientists found, spurred cells to accumulate excess centrosomes.

Cells that were induced to produce more Orc1, on the other hand, had the normal amount of centrosomes, even when centrosomes were induced to re-duplicate via drug treatment of cells. It was thus deduced that Orc1 allows cells to duplicate centrosomes once per division cycle, but prevents centrosomes from being re-duplicated.

This new role for Orc1 seems to be separate from its duties in helping cells copy DNA. The CSHL team found that a shortened version of Orc1 that lacked the ability to start DNA duplication was still able to limit centrosome copying to once per cell-division cycle.

Orc1 forces new centrosomes to stay in touch

Within each centrosome are a pair of tiny machines called centrioles. These duplicate during cell division to produce two centriole pairs.

Stillman's laboratory found that Orc1 also controls the number of centrioles in a cell. Before a pair is copied, the two centrioles normally stay connected to each other. Upon the cell's commitment to cell division, however, the centriole pair is duplicated to produce two new centriole pairs; this occurs precisely as copying of the chromosomes gets under way.

Stillman's team hypothesizes that it is this "engagement" of the paired centrioles that stops the original centriole pair from duplicating. In cells that lacked Orc1, the CSHL scientists found that the centrioles were "disengaged" from the original, suggesting that Orc1 might prevent re-duplication by helping the new centrosomes to stay connected to the old.

This function of Orc1 depends on its ability to physically associate with the centrosomes, the researchers showed. They suggest that Orc1 is ferried to the centrosomes by the action of a protein known as Cyclin A. This protein is found at high levels in cells at the start of the division cycle and helps cells make one copy of their DNA.

But a related protein called Cyclin E may be the target of Orc1. Cyclin E, which was also found to associate with Orc1, is known to be required for centriole and centrosome duplication and also stimulates the duplication of DNA in chromosomes. Orc1 antagonizes Cyclin E activity so that it duplicates centrosomes but cannot re-duplicate them.

The scientists thus propose that Orc1 enforces the number of centrosome copies by moving to centrosomes during the short temporal window in the cell division cycle when Cyclin E is still present in the cell. "During this time, if the effects of Cyclin E activity aren't counteracted by Orc1, centrosome re-duplication can occur," explains Stillman.

"I also think that this discovery suggests an ancient link between the

processes that duplicate DNA and the processes that separate the DNA in cells before cell division," he added.

"Orc1 controls centriole and centrosome copy number in human cells" will appear in the February 6th issue of *Science*. The full citation is: Adriana S. Hemerly, Supriya G. Prasanth, Khalid Siddiqui and Bruce Stillman. This article is available online at www.sciencemag.org. Bruce Stillman is President of the Laboratory in addition to running his own research laboratory.

Source: Cold Spring Harbor Laboratory