Study yields secrets of chromosome movement

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Investigators at St. Jude Children's Research Hospital have used the lowly yeast to gain insights into how a dividing human cell ensures that an identical set of chromosomes gets passed on to each new daughter cell. Errors in this critical part of cell division can cause one daughter cell to get extra copies of some chromosomes that should have moved into the other daughter cell, or no copies of other chromosomes—a problem that is prevalent in cancer and can cause miscarriages or disease, such as Down syndrome.

St. Jude researchers made their discovery by tracking the activity of a small army of molecules with exotic names like argonaute (Ago1) and dicer; these molecules help maintain a specialized, tightly packaged form of DNA called heterochromatin at the part of the chromosome called the centromere.

The investigators also showed the order in which certain critical events occur in setting up and maintaining this heterochromatin. The work is important because it gives scientists insight into how each daughter cell receives the normal number of chromosomes; and it offers important clues to understanding the genetic cause of certain catastrophic diseases. A report on this work appears in the May 25 issue of *Molecular Cell*.

All of the cell's DNA is wrapped around a series of structures, called histone octamers, to generate chromatin—much like thread wound around a spool. This chromatin is then further compacted to form the characteristic, thick structures commonly recognized in illustrations and

photographs as chromosomes. At the centromere, DNA is packaged into an even more compact and specialized form of chromatin called centromeric heterochromatin.

The centromere is the last point at which the two identical chromosomes are joined before the cell divides. Centromeric heterochromatin helps to yoke together the “sister chromatids” of each chromosome pair as they line up in the center of the dividing cell before separating and moving into their respective daughter cells. When the cell has ensured that it is safe to continue dividing, each sister chromatid moves in opposite directions toward the two new daughter cells that are forming.

“The cell must establish and then maintain centromeric heterochromatin to ensure that each chromosome pair is stable and securely linked together until it’s time to separate,” said Janet Partridge, Ph.D., assistant member of the St. Jude Department of Biochemistry. “Otherwise, the chromosome pairs would drift apart and leave daughter cells with too many or too few chromosomes.” Partridge is the report’s senior author.

The St. Jude team studied combinations of molecules in yeast called the RITS and RDRC complexes, which together with an enzyme called Clr4 (Suv39 in humans), establish and maintain centromeric heterochromatin in the yeast cell during a carefully choreographed series of steps.

RITS is composed of the proteins Ago1, Tas3 and Chp1 and works closely with RDRC. RDRC produces a type of genetic material called double-stranded RNA, which an enzyme, called dicer, then chops into smaller pieces called small interfering RNA (siRNA). siRNA is bound by RITS, and in turn, helps RITS to reinforce the centromeric heterochromatin and keep it stable.

In addition, the Clr4 enzyme puts chemical tags onto the histone “spool” in a process called methylation. Methylation attracts a protein called

Swi6 (HP1 in humans) to the chromosome to reinforce heterochromatin.

Previously, scientists—including Partridge's team—showed that cells lacking any component of RITS, RDRC or the Clr4 complex fail to assemble intact centromeric heterochromatin and suffer loss of chromosomes. However, researchers did not know whether the same components of these complexes are needed to support both establishment and the maintenance of centromeric heterochromatin. Therefore, Partridge's team developed specially modified yeast cells that allowed them to study these events individually.

By separating the Ago1 component of the RITS complex away from the Chp1-Tas3 components, rather than completely removing Ago1 from the cell, the researchers were able to generate yeast that could still maintain heterochromatin it had already established. These yeast cells had normal chromosome movement during cell division.

The investigators then showed that the same yeast cells also require the continuous presence of Clr4 to initially assemble normal centromeric heterochromatin. Specifically, the researchers used these yeast cells to show that the establishment of centromeric heterochromatin requires Clr4 to methylate histones at the centromere. The methylated centromere then attracts the RITS complex to this site. The St. Jude researchers' work also suggests that the siRNA is not so important for this first step, but does play an important role in propagating and maintaining centromeric heterochromatin.

"Until we did this study, it was virtually impossible to figure out which molecular events were specifically required for the two different processes of establishing and maintaining centromeric heterochromatin," Partridge said. "Now we have the tools to ask what is required for the cell to perform each task. This has important implications not just for understanding how centromeric heterochromatin assembles, but also for

learning how heterochromatin forms elsewhere on the chromosome, a process that is often disturbed in cancer."

Source: St. Jude Children's Research Hospital