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New Technique Explores the 'Black Holes' of Chromosomes

A new technique developed by Howard Hughes Medical Institute researchers significantly reduces the time required to isolate genetic material from the so-called “black holes” of chromosomes—the centromeric regions where one chromosome is joined to its sister in the cell.

Most scientists believe that the centromeric regions of chromosomes are likely to hold invaluable clues to understanding the machinery that powers cell division. But those clues have remained largely obscure because the centromeres contain vast, confusing stretches of repetitive DNA sequences and segments that shift position in the genome.

According to the researchers, the new technique will speed the process of understanding the function of centromeric regions by enabling the rapid comparison of these regions of DNA among different plants and possibly even mammals. Such studies may help researchers understand the evolution—and thus the function—of the centromeric regions.

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The technique may also enable creation of “designer chromosomes” in crop plants that could alter their characteristics to produce desirable traits, according to Howard Hughes Medical Institute investigator Daphne K. Preuss. She and her colleagues at the University of Chicago published a research article describing the technique in the October 2004 issue of the journal *Nature Methods*.

Preuss's team reported that they applied the technology to isolate centromeric DNA from the plant *Arabidopsis thaliana*, a member of the mustard family widely used as a model plant in biology.

“The centromeric regions perform a number of essential functions,” said Preuss. “They include directing inheritance of a chromosome and keeping the sister chromatids bound to each other until the later stages of meiosis and mitosis.” Meiosis is the cell division process that, in the case of plants, results in the generation of pollen and egg cells; in humans, the process generates sperm and oocytes. Mitosis is the cell division process by which organisms increase the number of cells in development and maintenance. The centromeric region also includes genes important for preserving the vital function of the region in cell division.

“Despite their importance, the DNA sequencing projects have often been very challenged in obtaining complete DNA sequence assemblies in the centromeric region because they are highly repetitive,” said Preuss. For example, she said, the project to sequence the *Arabidopsis* genome took three years of arduous work by a group of laboratories to develop sequence information for the plant's centromeric DNA.

To speed that process, Preuss and her colleagues developed an isolation technique that exploits the fact that centromeric DNA is distinguished by a high number of methyl groups, which attach to the cytosine base pairs contained in DNA. Methylation is one method by which the cell maintains genome regions in an inactive state.

“Our approach was the opposite of what a lot of genome projects do, because they try to focus on the genes and exclude the repetitive DNA,” said Preuss. “We wanted to selectively identify the highly repetitive DNA that corresponds to the centromere.”

To identify those regions selectively, the researchers used an enzyme to snip apart the plant's genome, but did not cut the DNA in methylated regions. By using these resistant fragments to probe the *Arabidopsis* genome, the researchers could determine whether they had identified centromeric DNA. The studies were highly accurate in identifying centromeric DNA, said Preuss.

“It was a very exciting result,” she said. “We had spent three years slogging through the centromeres using traditional methods, which involve sequencing small regions and using them as probes to ‘walk’ along the DNA to identify others. But with this method, the results just popped up in three days.”

Once the researchers had identified the centromeric regions in *Arabidopsis*, they extended the technique to isolate such regions in three other plant species that shared a common ancestor with *Arabidopsis*. The method succeeded, even though these plants had different chromosomal numbers and genome sizes than *Arabidopsis*.

“We wanted to know if this was a general methodology,” said Preuss. “Since these other species were evolutionarily separated from *Arabidopsis* by

different times, it gave us a beginning in understanding how centromeres evolved.”

The success of the technique with the other species demonstrates that rapid analysis of the centromeric regions in a broad range of plants, and even mammals, is feasible. “We believe that this technique could be extended to mammals, because the one thing that plants and mammals have in common about their centromeres is the extensive heavy methylation of centromeric regions,” she said. “It’s certainly worth trying, given that it only takes three days.”

Analysis of the plant centromeres could have important agricultural applications, said Preuss. “Having a good understanding of centromeres means that you can actually make designer chromosomes,” she said. Such chromosomes could drastically alter a plant’s characteristics to create improved crops plants, said Preuss.

“In terms of clinical applications, we do know that there are inherited disorders and cancers associated with centromere function, so that understanding of how the centromere works could offer important insights into those disorders,” Preuss added.